

β -Lactamase inhibitors- the next generation....and beyond?

What is new and why they work!

Robert A. Bonomo, MD

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Case VA CARES

Case Western Reserve University School of Medicine

It takes a village.....

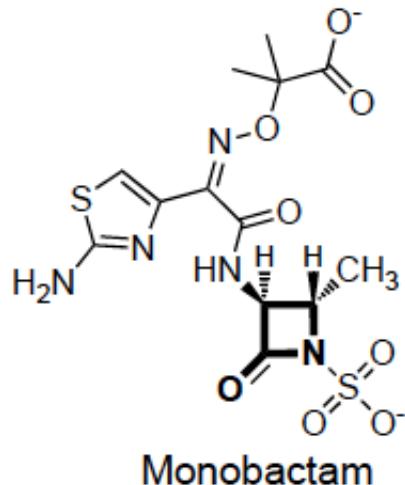
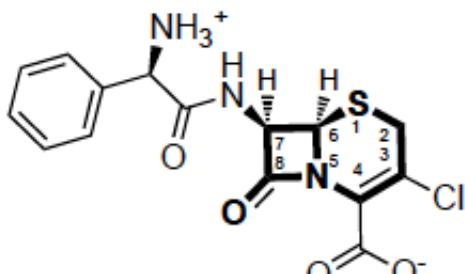
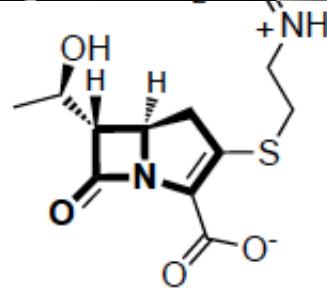
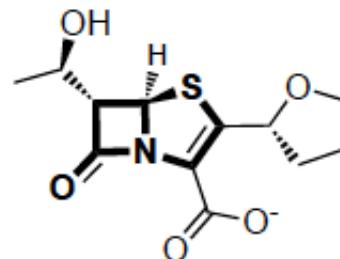
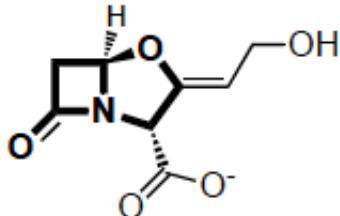
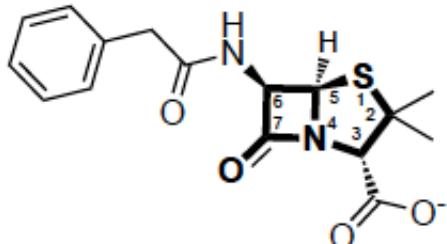
- VA Merit Review (Dr. Jane Battle), NIH (Drs. J. Gi, Dixon, Ernst, Knisely and Eakin), ARLG, Harrington Foundation. Dr. Neal Peachey and Holly Henry. Dr. WH Boom
- Professor Keith Kaye
- Drs. Karen Bush, Louis B. Rice, and David M. Shlaes
- My LAB- Ms. Kris Hujer and Andrea Hujer; Mr. Chris Bethel, Steve Marshal, Nick Domitrovic, Sue Rudin; Dr. Maria Fernanda Mojica, Magda Taracila, Dr. Laura Rojas Coy
- Drs. Kris Papp-Wallace and Melissa Shelton Barnes
- “The KPC Club”: Drs. Federico Perez, M. Wright, Mark Adams, David van Duin, Keith Kaye, Cesar Arias, Michael Jacobs, Julia Segre, Scott Evans, Barry Kreiswirth, Liang Chen, Marcelo Tolmasky, Maria Soledad Ramirez, Latania Logan, Pranita Tamma, and Sandy Richter
- “The Psda Club” Andrew Mack, Drs. Shozeb Haider, John Dekker, George Drusano
- Drs. Vance Fowler, Focco van den Akker, Fabio Prati, Emilia Caselli, Marisa Winkler, Roberto Viau, Paul Carey, John Buynak, Brad Spellberg
- “The NDM Club” Drs. Alejandro Vila, Jim Spencer, Walter Fast, Mike Crowder, Rick Page, Seth Cohen, Rick Page
- “The NTM Club” Khald Dousa, Sebastian Kurz, Barry Kreiswirth
- AstraZeneca, Allergan, Merck, Wockhardt, GSK, Roche, Achaogen, Shionogi,

Outline

- The “State of Affairs”
- Working with Pharma in preclinical and clinical studies:
 - Avibactam
 - Relebactam
 - Vaborbactam
 - WCK5153 and Zidebactam
 - ETX2514
 - Nacubactam
- AAI101
- New compounds!

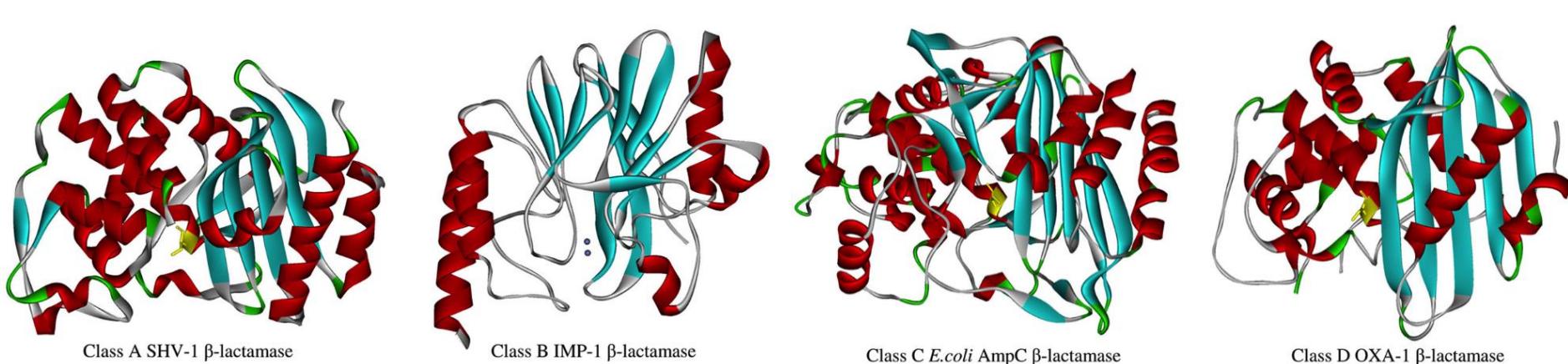
My goal is to provide the basic knowledge required to distinguish and differentiate each of the novel BLIs that are commercially available and in development

“Life-saving Therapy”

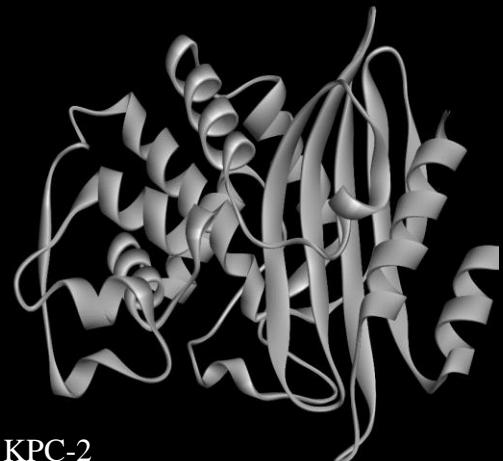


**Impact on society.
Why are these drugs losing their efficacy?**

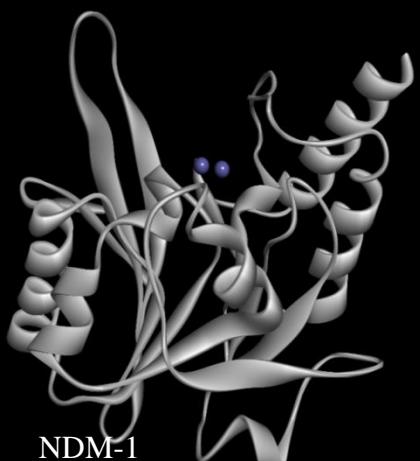
β -lactamases



And then....the carbapenemases



KPC-2



NDM-1

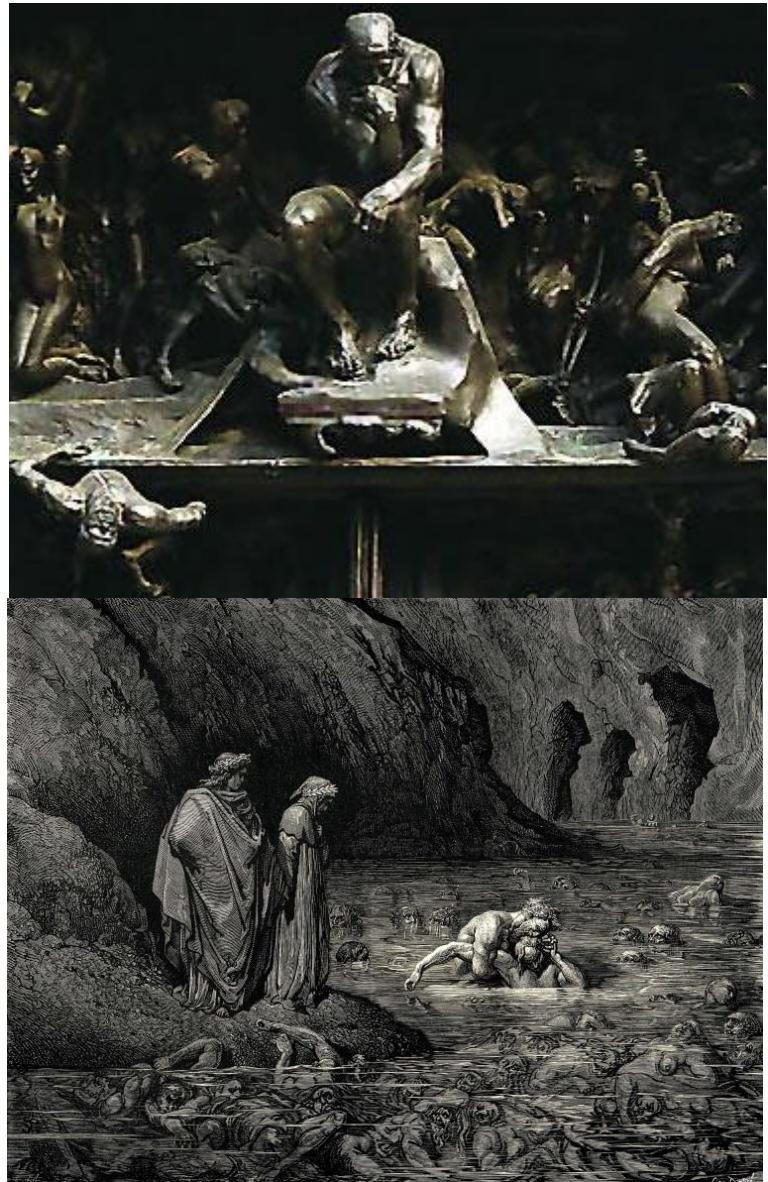


OXA-48

VIM-2

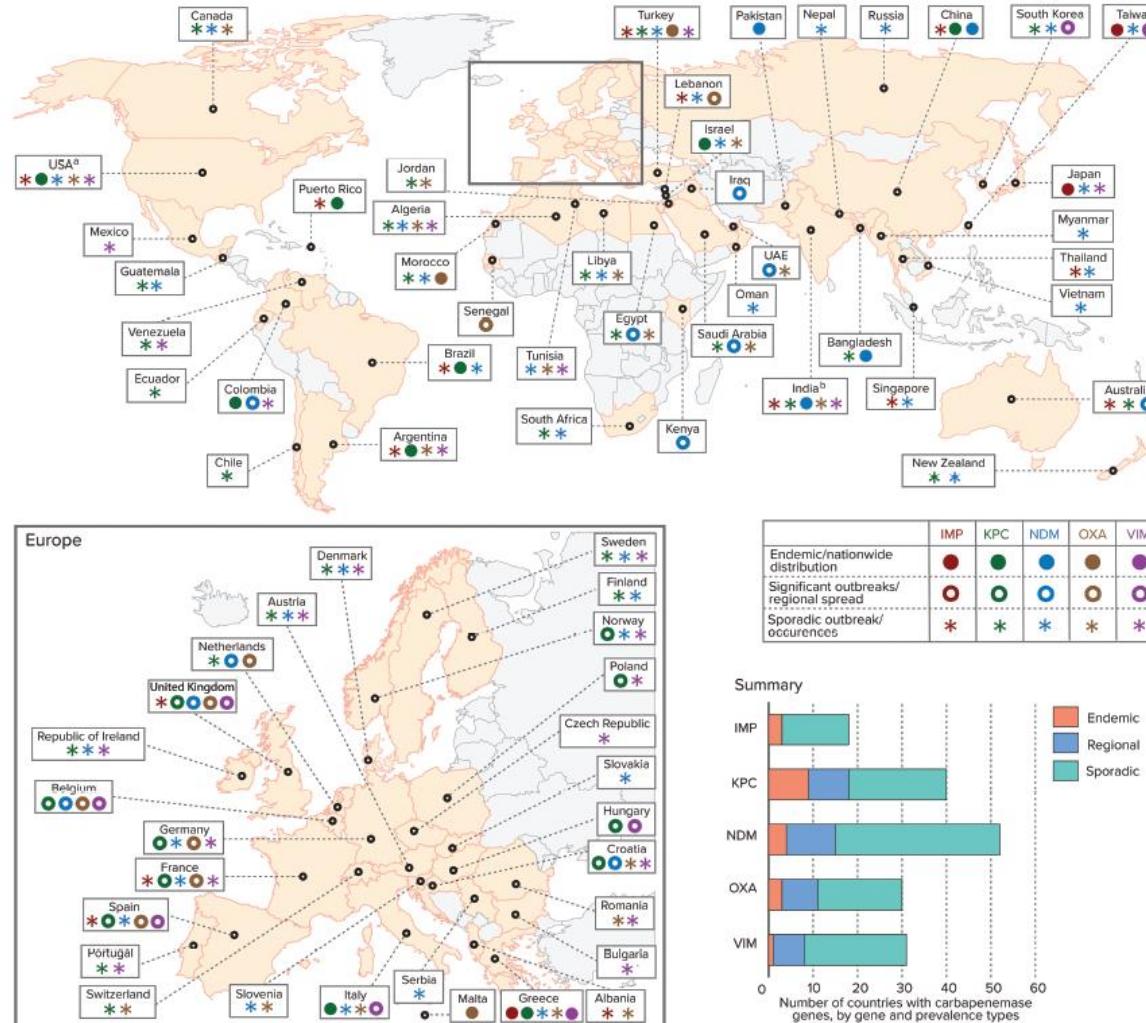


*"Inky darkness and horror...no longer can imipenem
save your life...."*



“Global Menace”

The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace

Latania K. Logan^{1,3} and Robert A. Weinstein^{2,3}

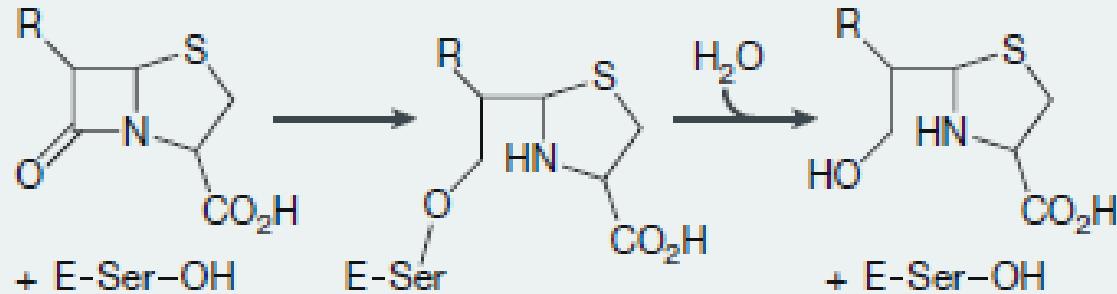
Need to embark upon a journey of discovery...



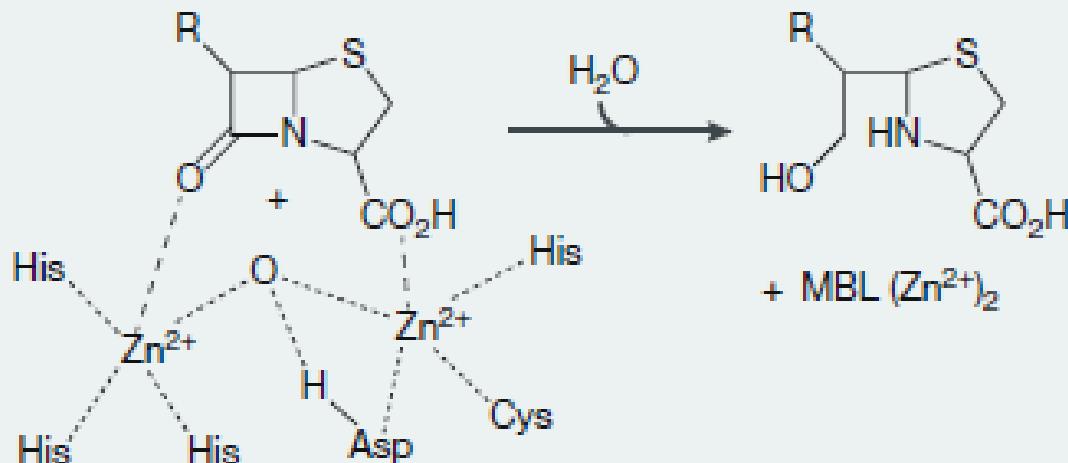
Cezanne

To overcome β -lactamases, we must understand the mechanism

Hydrolysis by a serine β -lactamase

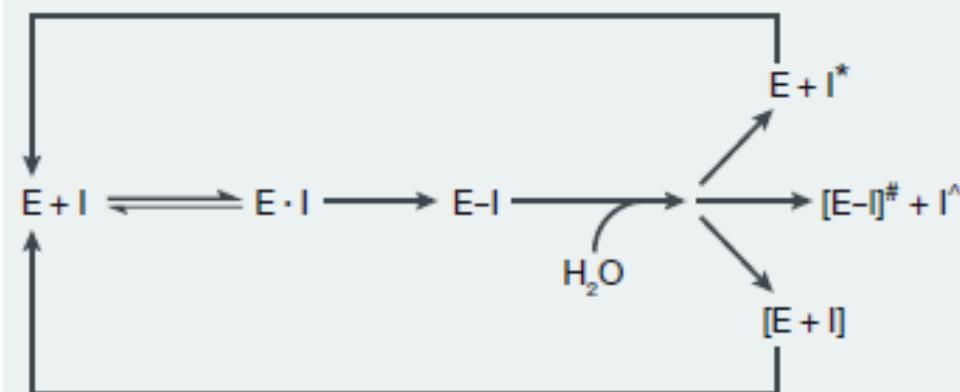


Hydrolysis by an MBL



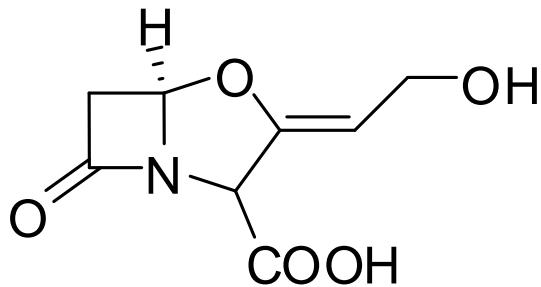
Interplay between β -lactamases and new β -lactamase inhibitors

Karen Bush¹* and Patricia A. Bradford²*

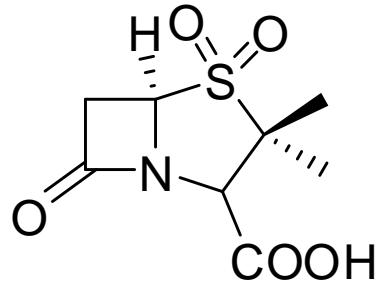




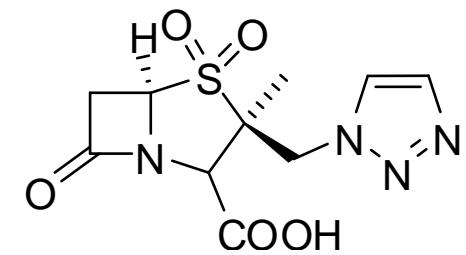
Inhibit β -lactamases: a major clinical achievement!



Clavulanic Acid



Sulbactam



Tazobactam

Olivanic acid

Sulfones

TABLE 3. Activity of β -lactamase-labile antibiotics in the presence of sodium clavulanate

β -Lactam antibiotic plus sodium clavulanate	Minimum inhibitory concn ($\mu\text{g}/\text{ml}$) ^a					
	<i>Staphylococcus aureus</i> Russell	<i>Klebsiella aerogenes</i> NCTC 418	<i>Proteus mirabilis</i> C889	<i>Escherichia coli</i> JT39	<i>Escherichia coli</i> JT410	<i>Pseudomonas aeruginosa</i> Dalglish
Sodium clavulanate alone	15	31	62-125	31	31	250
Ampicillin alone	500	250	>2,000	>2,000	250	>2,000 ^b
Ampicillin + 1 $\mu\text{g}/\text{ml}$	0.8	0.4	62	31	250	2,000 ^b
Ampicillin + 5 $\mu\text{g}/\text{ml}$	0.02	0.1	8	4	250	500 ^b
Ampicillin + 20 $\mu\text{g}/\text{ml}$					125	125 ^b
Cephaloridine alone	0.6		62	62	62	
Cephaloridine + 1 $\mu\text{g}/\text{ml}$	0.15		8	4	62	
Cephaloridine + 5 $\mu\text{g}/\text{ml}$	0.06		4	2	62	

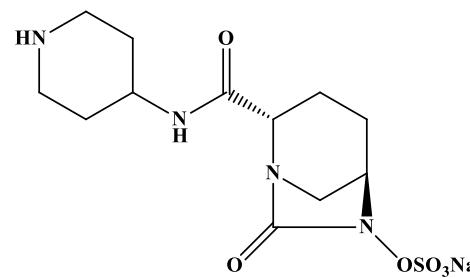
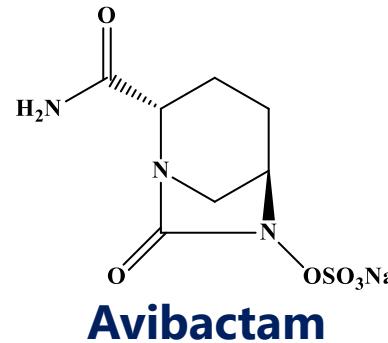
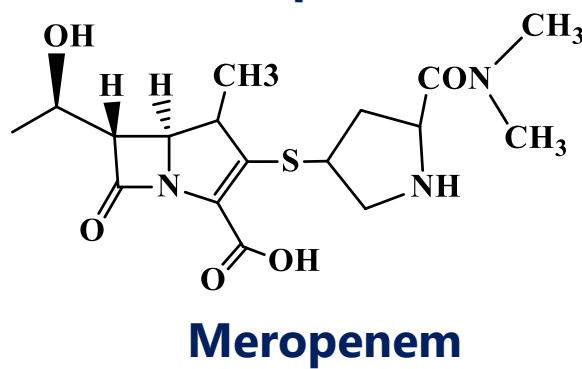
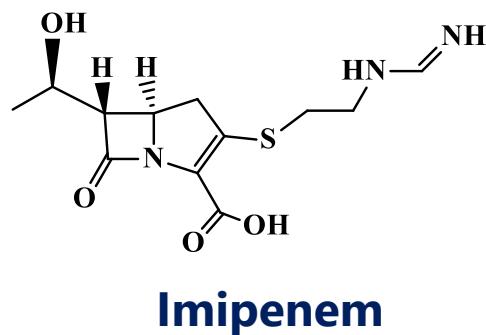
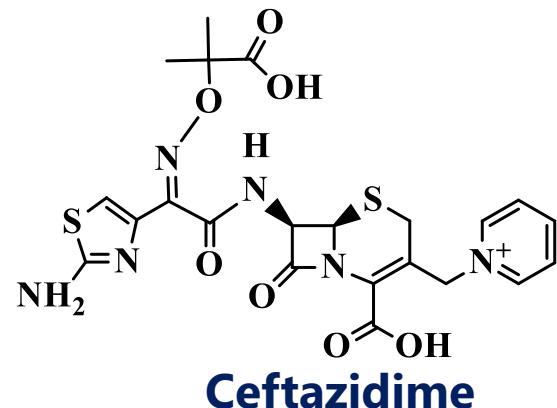
^a Microtiter technique using serial dilutions in tryptone soy broth with a 1/500 dilution of overnight broth culture as inoculum. End points read after 18 h at 37°C.

^b Carbenicillin in place of ampicillin.

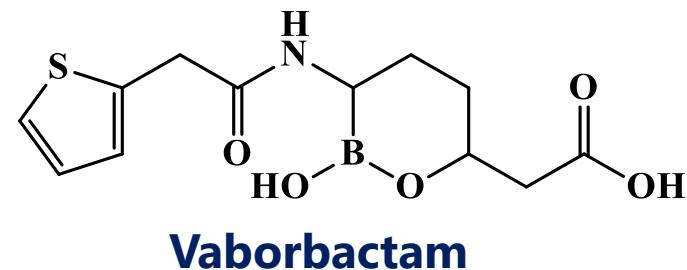


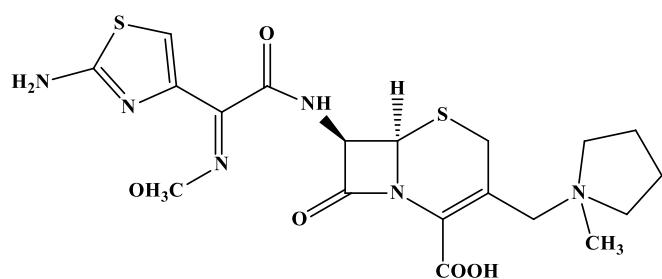
Augmentin
Timentin
Unasyn
Zosyn

Preserving the β -Lactam Promise –the “new generation of BLI”

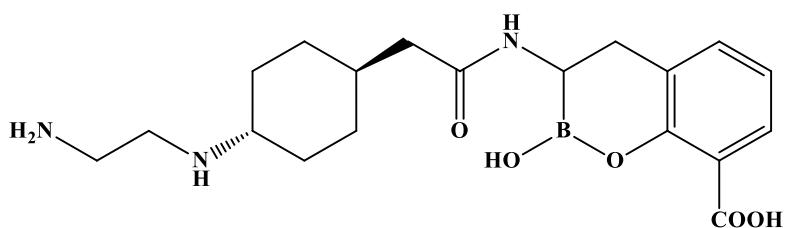


Relebactam

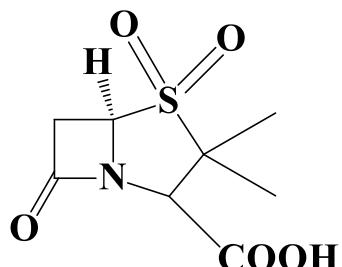




Cefepime



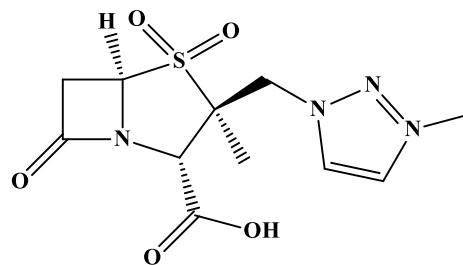
VNRX-5133



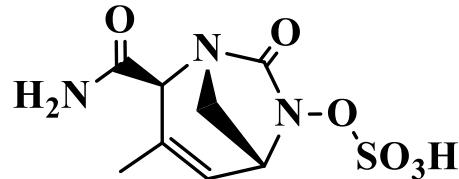
Sulbactam



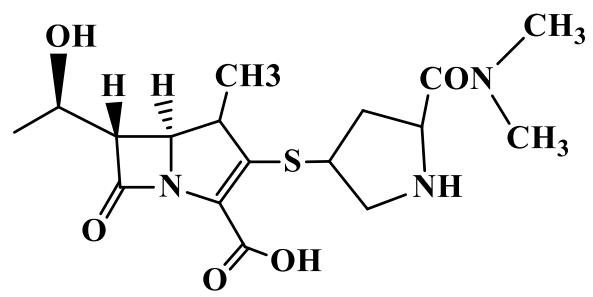
Zidebactam



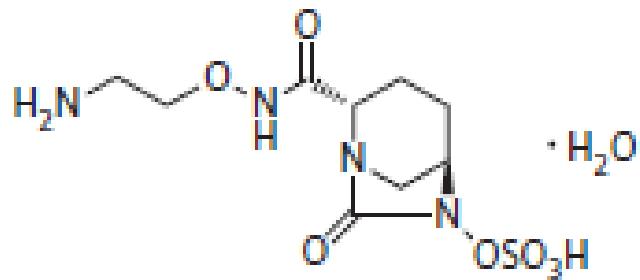
AAI101



ETX-2514



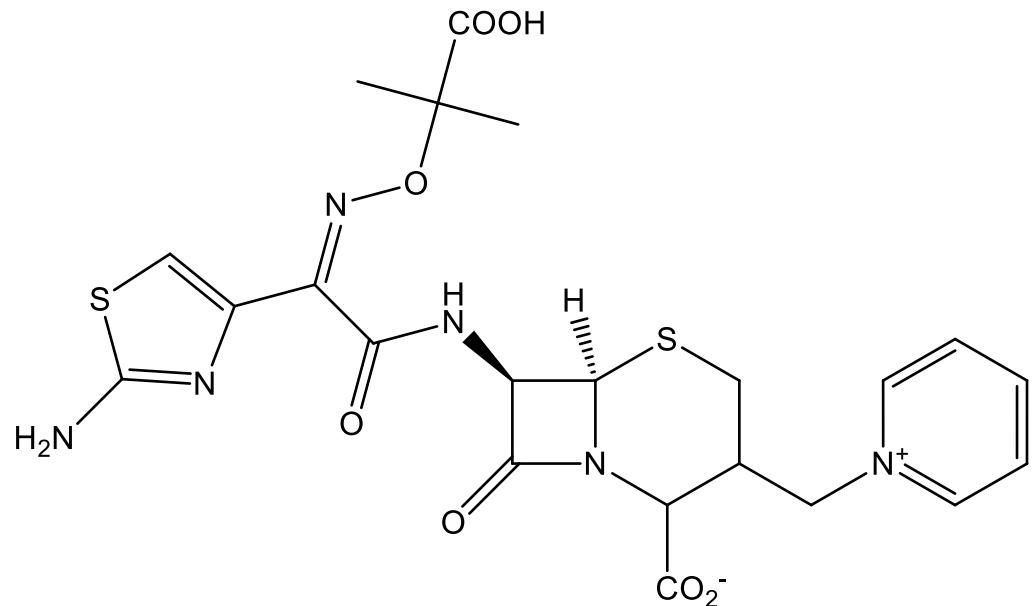
Meropenem



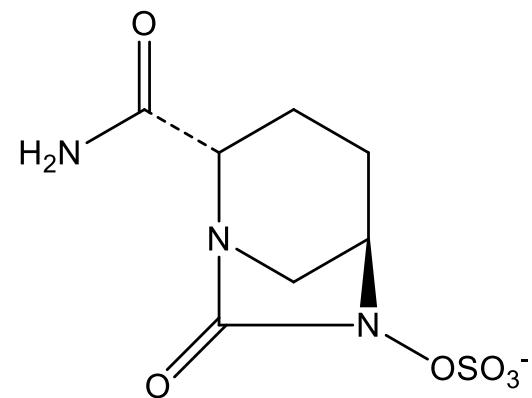
Nacubactam

AVIBACTAM

Ceftazidime



Avibactam



- **Avibactam resembles portions of the cephem bicyclic ring system,**

New β -Lactamase Inhibitors: a Therapeutic Renaissance in an MDR World

Against *Kp KPC*, the addition of AVI improves the activity of taz (~4x MIC reduction).

Sarah M. Drawz,^a Krisztina M. Papp-Wallace,^{b,c} Robert A. Bonomo^{b,c,d,e,f,g}

Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota, USA^a; Research Service, Louis Stokes Cleveland Department of Veterans Affairs, Cleveland, Ohio, USA^b; Departments of Medicine,^c Pharmacology,^d and Molecular Biology and Microbiology,^e Case Western Reserve University, Cleveland, Ohio, USA



TABLE 1 MICs of β -lactam and β -lactam-avibactam combinations against select pathogens^a

Pathogen	MIC ($\mu\text{g/ml}$) ^b					
	CAZ	CAZ-AVI	CPT	CPT-AVI	ATM	ATM-AVI
<i>K. pneumoniae</i> with OXA-48	256/512	0.25/0.5				
<i>K. pneumoniae</i> with CTX-M-15	8/64	0.06/0.25				
<i>K. pneumoniae</i> with KPC-2	$\geq 512/\geq 512$	0.25/1			$\geq 512/\geq 512$	$\leq 0.06/\leq 0.06$
<i>E. coli</i> with ESBL	16/64	0.12/0.25				
<i>E. coli</i> with AmpC	16/64	0.12/0.5				
<i>E. coli</i> with OXA-48	4	<0.008				
<i>E. coli</i> with IMP-1	256	64				
<i>Enterobacteriaceae</i> with multiple β -lactamases, including KPC-2			>64/>64	0.5/2		
<i>Enterobacteriaceae</i> with multiple β -lactamases, including AmpC			256/>256	0.5/2		
<i>Enterobacteriaceae</i> with VIM	64–512	64–512			0.25–256	0.12–0.5
<i>P. aeruginosa</i>	8/64	4/8	>64/>64	16/>32	16/32	8/32
<i>P. aeruginosa</i> with ESBL PER-1	128/128	4/16				
<i>A. baumannii</i>			>64/>64	32/>32		
<i>A. baumannii</i> with PER-1, OXA-51, and OXA-58	128/ ≥ 512	32/256				
<i>S. aureus</i>			1/2	1/2		

^a Data were adapted from references 15, 16, 19, 20, 21, and 24. Avibactam was added at 4 $\mu\text{g/ml}$. Abbreviations: CAZ, ceftazidime; AVI, avibactam; CPT, ceftaroline; ATM, aztreonam.

^b Numbers separated by a forward slash indicate MIC_{iso}/MIC_{co} values. Empty cells indicate that values were not reported.

Ceftazidime-Avibactam

Pathogen	Antibiotic MIC ₅₀ / MIC ₉₀ (US Hospitals; 2011-4)			
	CAZ-AVI _{4ug/ml}	Ceftazidime	Meropenem	Pip/Tazo
Enterobacteriaceae	0.12 / 0.25	0.12 / 8	≤ 0.06 / ≤ 0.06	2 / 16
CTX-M-15 like	0.12 / 0.5	16 / >32	≤ 0.06 / ≤ 0.06	8 / >64
KPC-Producing Enterobacteriaceae	0.25 / 1	>32 / >32	> 8 / > 8	>64 / > 64
<i>P. aeruginosa</i>	2 / 8	2 / 32	0.5 / 8	4 / 64
CAZ MIC ≥ 8	4 / 16	≥ 8 / ≥ 8	4 / >32	64 / > 64
MER MIC ≥ 4	4 / 16	16 / >32	≥ 4 / > 4	32 / > 64

- Inhibited by class B and some class D B-lactamases or other mechanisms of bacterial protection (e.g. efflux pumps, porin size, cell wall alteration)
- **Gaps in coverage: *K. pneumoniae* producing MBLs (VIM, MDM), ESBL-Acinetobacter, Burkholderia, Stenotrophomonas (NDM), *Pseudomonas* spp. with ceftaz-efflux pumps, anaerobes, MRSA**

Ceftazidime/avibactam versus standard-of-care agents against carbapenem-resistant Enterobacteriaceae harbouring *bla*_{KPC} in a one-compartment pharmacokinetic/pharmacodynamic model

Katie E. Barber^{1*}, Jason M. Pogue², Henderson D. Warnock¹, Robert A. Bonomo^{3–5} and Keith S. Kaye⁶

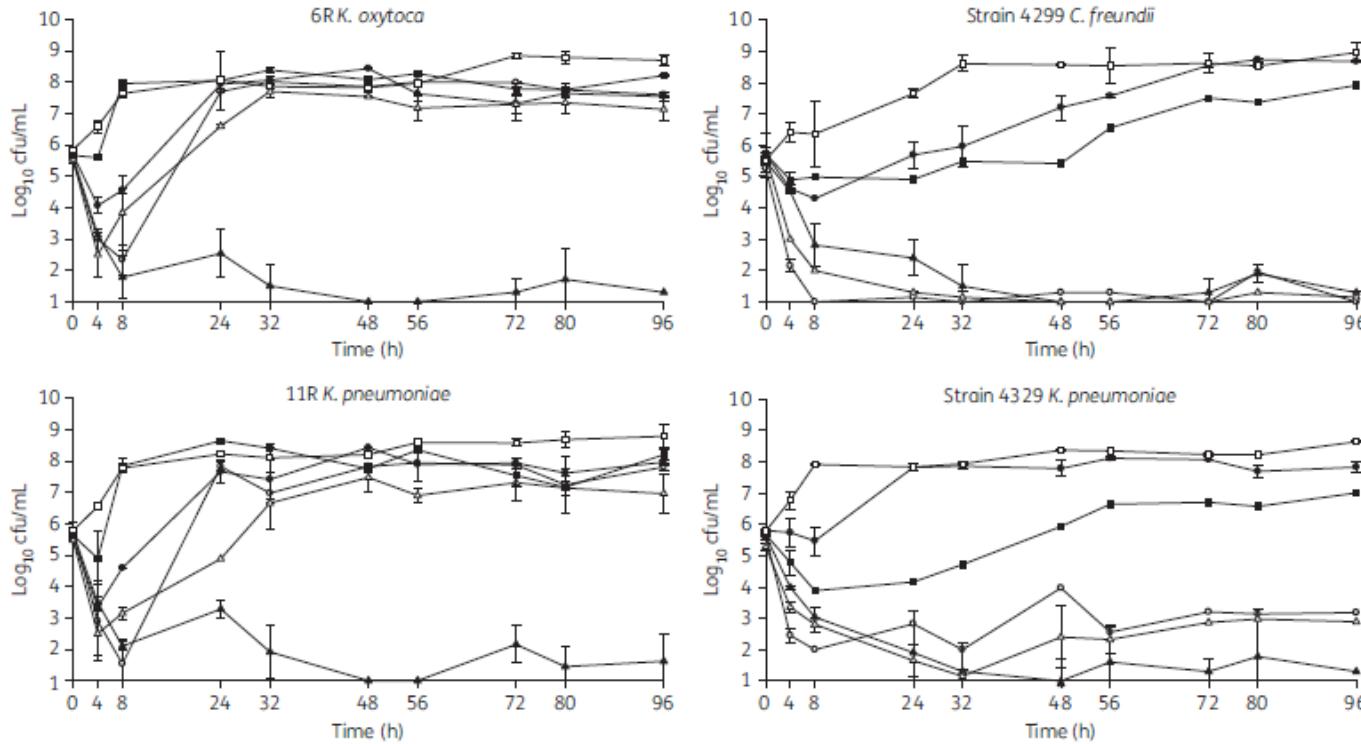


Figure 1. PK/PD graphs for four study pathogens in in vitro models. Open circles, amikacin; filled triangles, ceftazidime/avibactam; filled circles, meropenem; open triangles, polymyxin B; filled squares, tigecycline; open squares, drug-free growth control.

1. 2.5 g taz avi vs 1.25 mg/kg polymyxin
2. Compared to Polymyxin, taz avi did better against different strains

Inhibition of *Klebsiella* β -Lactamases (SHV-1 and KPC-2) by Avibactam: A Structural Study

Nikhil P. Krishnan^{1*}, Nhu Q. Nguyen^{1*}, Krisztina M. Papp-Wallace², Robert A. Bonomo^{1,2,3,4,5}, Focco van den Akker^{1*}

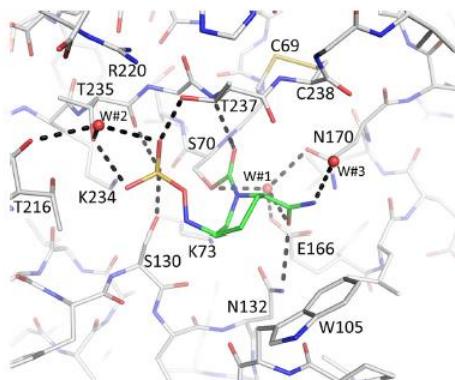


Fig 4. Interactions of avibactam in the active site of KPC-2. Avibactam is shown with green carbon atoms. Hydrogen bonds are depicted as dashed lines (cut-off distance is 3.2 Å). The deacylation water is present (labeled W#1). Additional waters are labeled W#2–3.

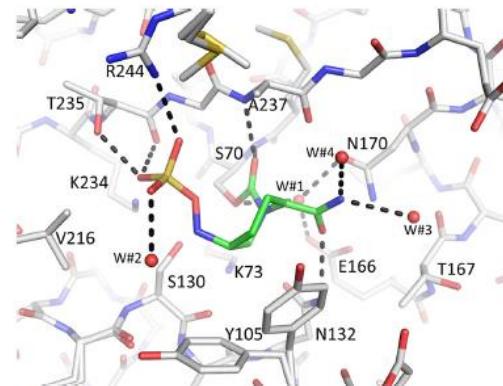
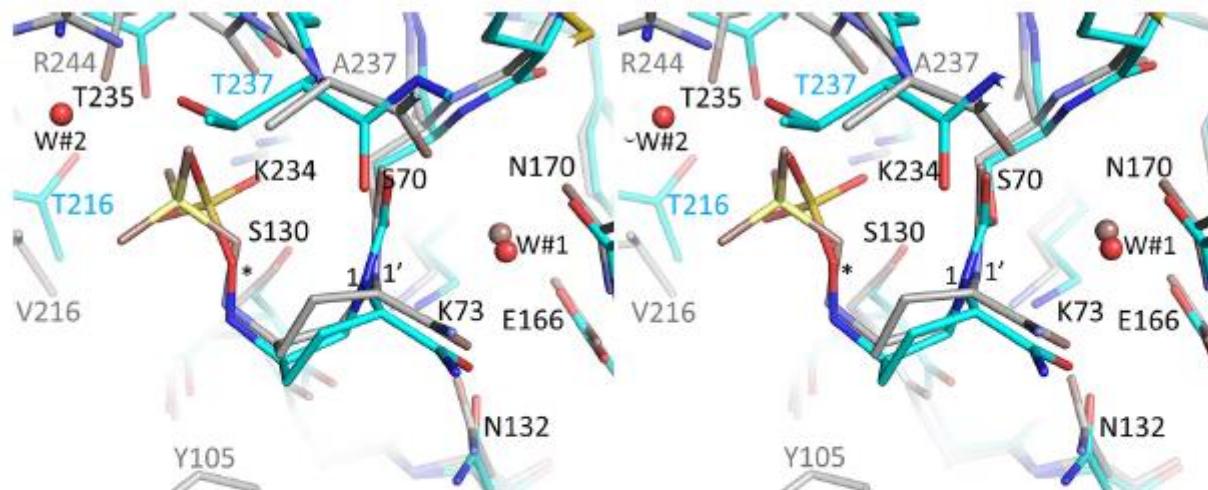


Fig 5. Avibactam in the active site of SHV-1. Interactions of avibactam (shown with green carbon atoms) in the active site of SHV-1. Hydrogen bonds are depicted as dashed lines. The deacylation water is present (labeled W#1). Additional waters are labeled W#2–4.



Active site of KPC is broader and “more open”

Colistin vs. ceftazidime –avibactam in the treatment of Infections due to Carbapenem-resistant Enterobacteriacea

David van Duin, et al
Clin Infect Dis. 2018 Jan 6;66(2):163-171

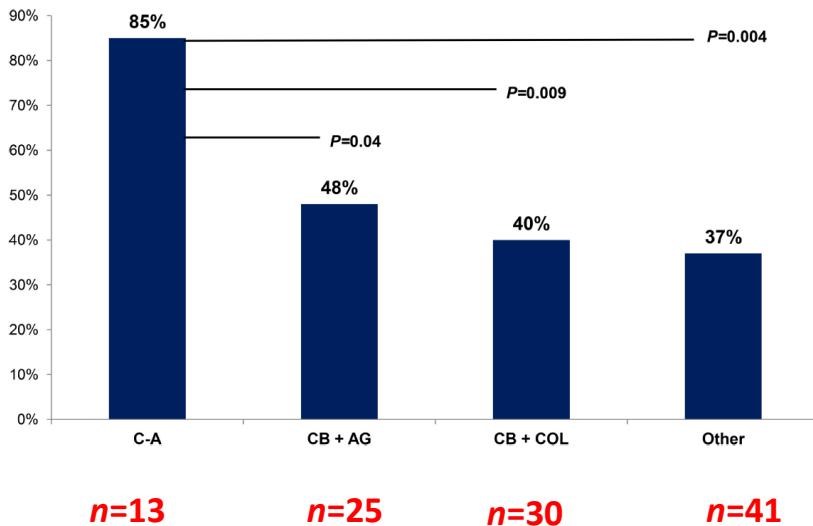
CRACKLE-I

- 137 patients met criteria; 38 patients were treated first with ceftazidime-avibactam and 99 with colistin.
- BSI (n=63, 46%) > PNA(n=30, 22%).
- No isolates had *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP} or *bla*_{OXA-48}.
- ST258A (18/54, 33%) and ST258B (23/54, 43%) were the most commonly encountered clades of CRKP

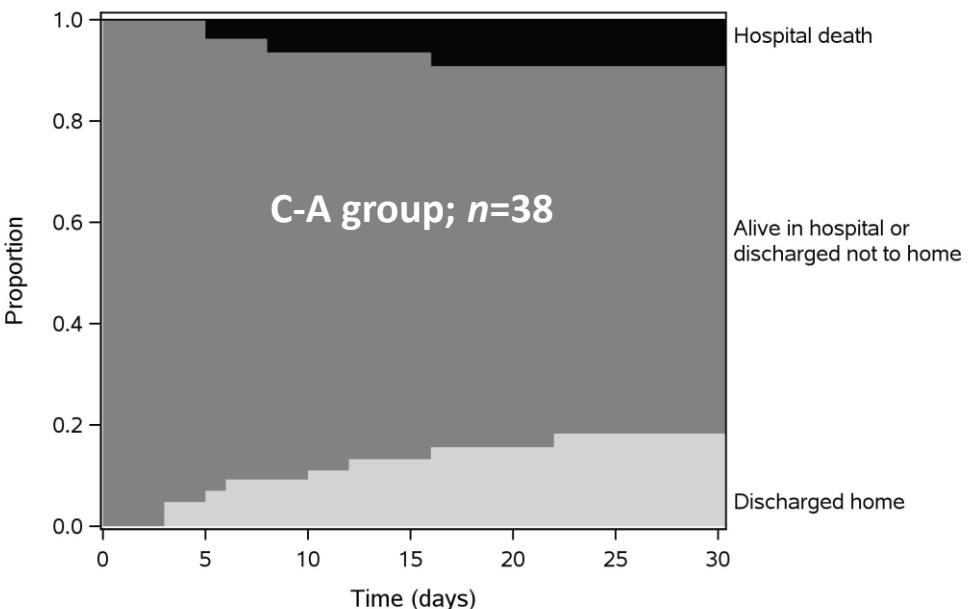
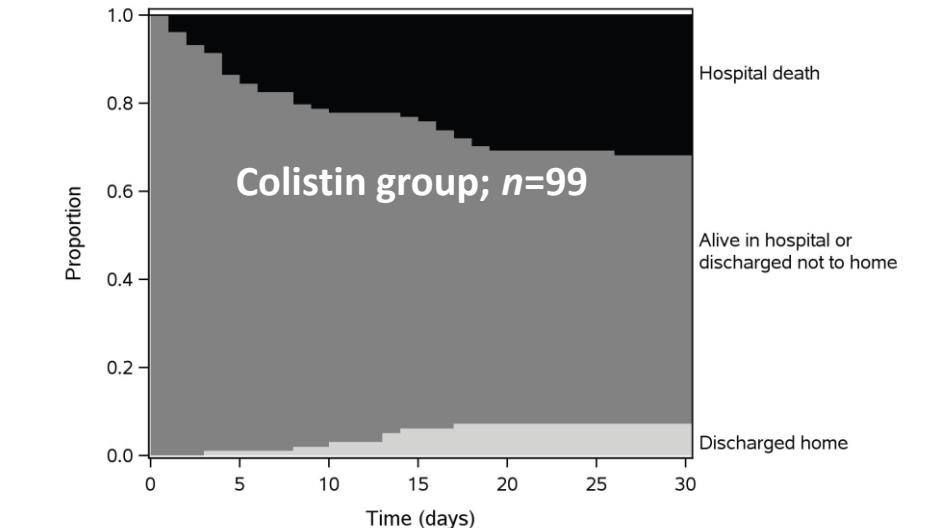
Consortium on Resistance Against Carbapenems in Klebsiella and other Enterobacteriaceae (CRACKLE), a prospective, multicenter, observational study.

Conclusions

- In patients treated with TAZ AVI vs. colistin all-cause hospital mortality at 30-days after starting treatment was 9% vs 32%
- Thus....In this prospective, observational, multi-center cohort, all-cause propensity adjusted mortality was decreased in patients with CRE infections started on ceftazidime/avibactam vs. colistin (absolute risk reduction 23% [95% CI 9%-35%], p=0.0012).



***“One good drug
is better than
two bad ones”***



Probing the Mechanism of Inactivation of the FOX-4 Cephamycinase by Avibactam

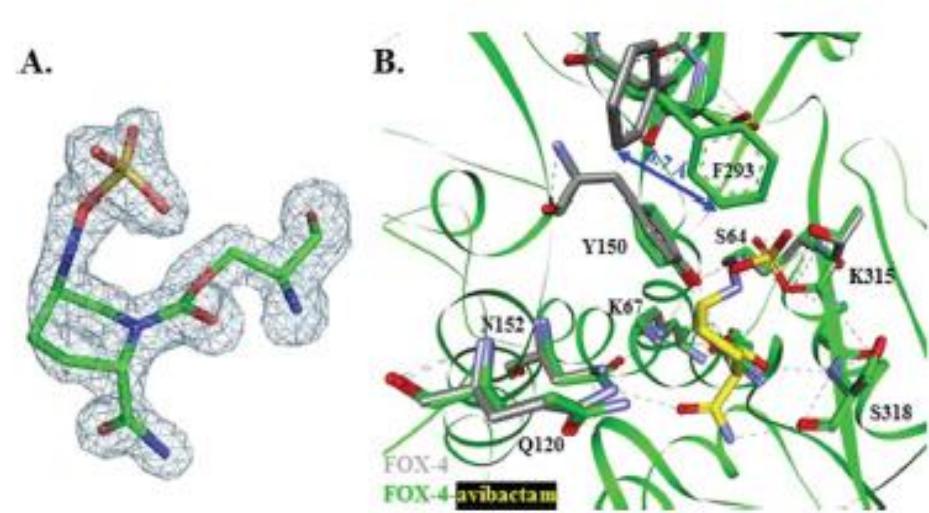
Michiyoshi Nukaga,^a Krisztina M. Papp-Wallace,^{b,c,d} Tyuji Hoshino,^a Scott T. Lefurgy,^f Christopher R. Bethel,^b Melissa D. Barnes,^{b,c} Elise T. Zeiser,^b J. Kristie Johnson,^g Robert A. Bonomo^{b,c,h,i}



FIG 2 Worldwide distribution of FOX β -lactamases (black).

Flexible, highly mobile Ω loop

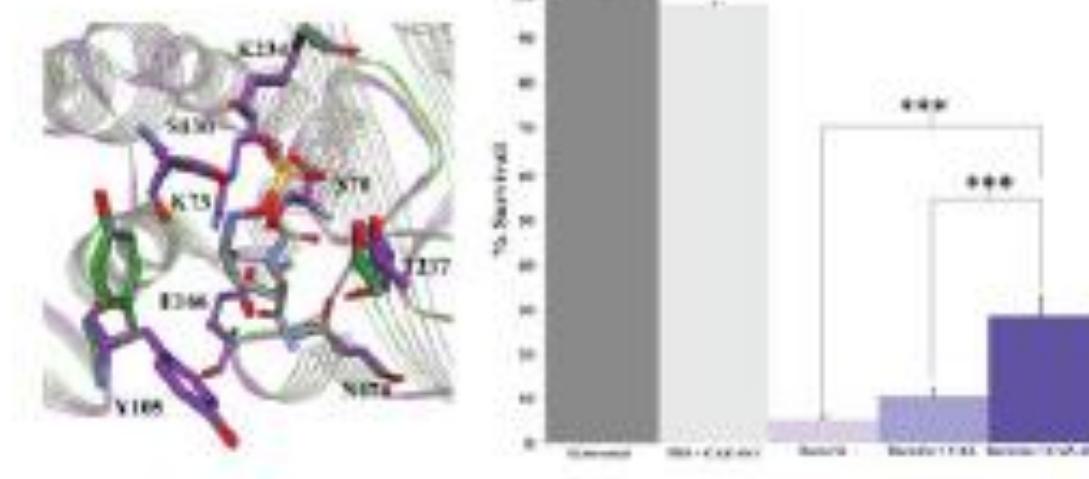
More than just
Pseudomonas



Overcoming an Extremely Drug Resistant (XDR) Pathogen: Avibactam Restores Susceptibility to Ceftazidime for *Burkholderia cepacia* Complex Isolates from Cystic Fibrosis Patients

Krisztina M. Papp-Wallace^{†‡¶**Γ}, Scott A. Becka[†], Elise T. Zeiser[†], Nozomi Ohuchi[§], Maria F. Mojica^{†¶}, Julian A. Gatta[†], Monica Falleni[‡], Delfina Tosi[‡], Elisa Borghi[‡], Marisa L. Winkler^{†#}, Brigid M. Wilson[†], John J. LiPuma[⊗], Michiyoshi Nukaga[§], and Robert A. Bonomo^{†‡#¶.⊥.Π.**Γ}

Formed the
basis of a
novel
therapy for
terminal
infections in CF



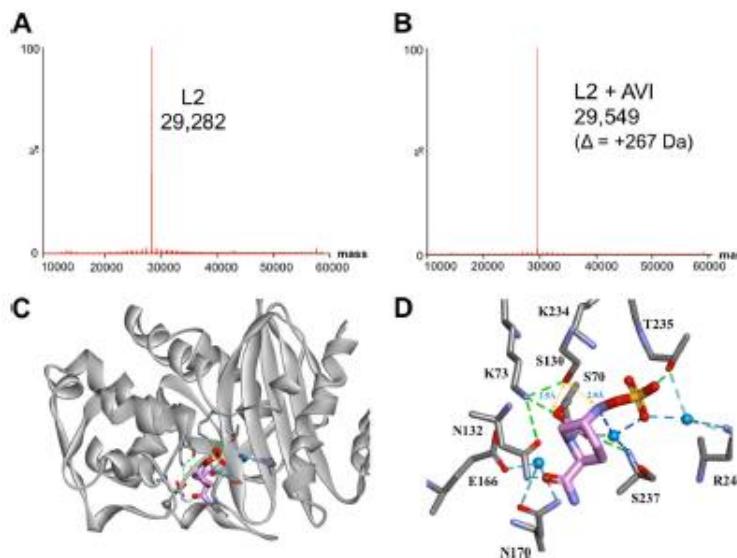
Avibactam Restores the Susceptibility of Clinical Isolates of *Stenotrophomonas maltophilia* to Aztreonam

Maria F. Mojica,^{a,b} Krisztina M. Papp-Wallace,^{a,b,c} Magdalena A. Taracila,^{b,c} Melissa D. Barnes,^{b,c} Joseph D. Rutter,^b Michael R. Jacobs,^{d,e} John J. LiPuma,^f Thomas J. Walsh,^g Alejandro J. Vila,^{h,i} Robert A. Bonomo^{a,c,f,k,l}

TABLE 1 Steady-state kinetic parameters of L2 with NCF and AVI^a

Parameter	Value
NCF K_m (μM)	62 ± 4
NCF k_{cat}/K_m ($\mu\text{M}^{-1} \cdot \text{s}^{-1}$)	9.14 ± 0.01
AVI $K_{i,app}$ (μM)	0.66 ± 0.07
AVI k_2/K ($\text{M}^{-1} \cdot \text{s}^{-1}$)	$47,000 \pm 131$
AVI k_{off} (s^{-1})	0.0015 ± 0.0001
AVI $k_{off} t_{1/2}$ (min)	4.0 ± 0.2

^aNCF, nitrocefin. Values reported are averages \pm standard deviations from triplicate experiments.



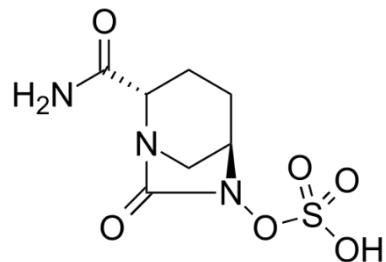
RECAPTURE

Ceftaz-avi vs Doripenem cUTI/AP

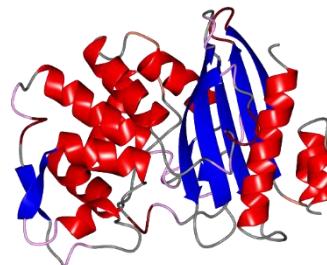
	Caz-avi N = 393	Doripenem N = 417	Tx Diff (95% CI)
	n (%)		
Day 5 sx resolution	276 (70.2)	276 (66.2)	4.0 (-2.39, 10.42)
TOC clin/micro	280 (71.2)	269 (64.5)	6.7 (0.30, 13.12)
TOC micro (EMA)	304 (77.4)	296 (71.0)	6.4 (0.33, 12.36)
Ceftaz NS, n/n (%)	48/75 (64.0)	51/85 (60.0)	4.0 (-11.11, 18.81)

- Caz-avi 2000/500mg q 8 hrs vs doripenem 500mg q 8h; po switch p 5 d
- Caz-avi superior in micro response at TOC (EMA)**
- Adverse events similar

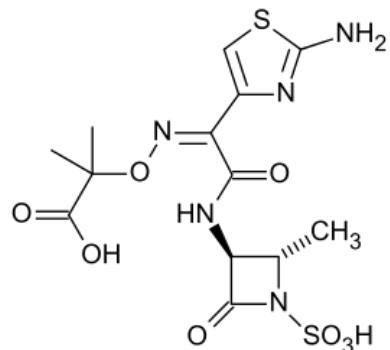
Hypothesis



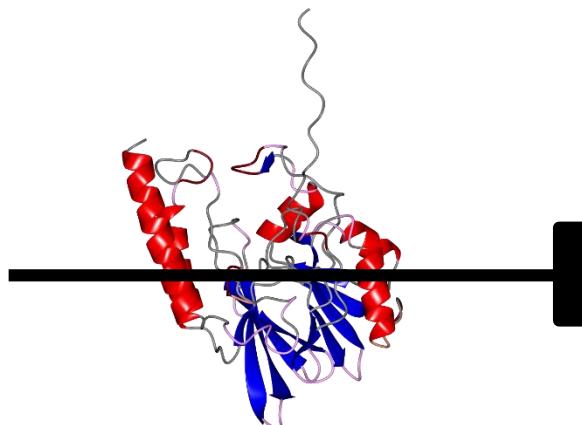
Avibactam



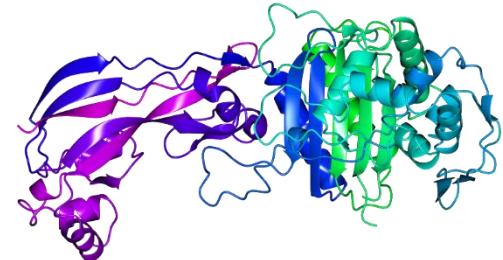
L2



Aztreonam



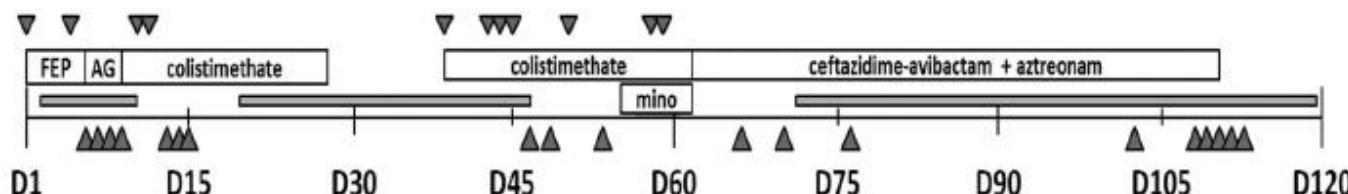
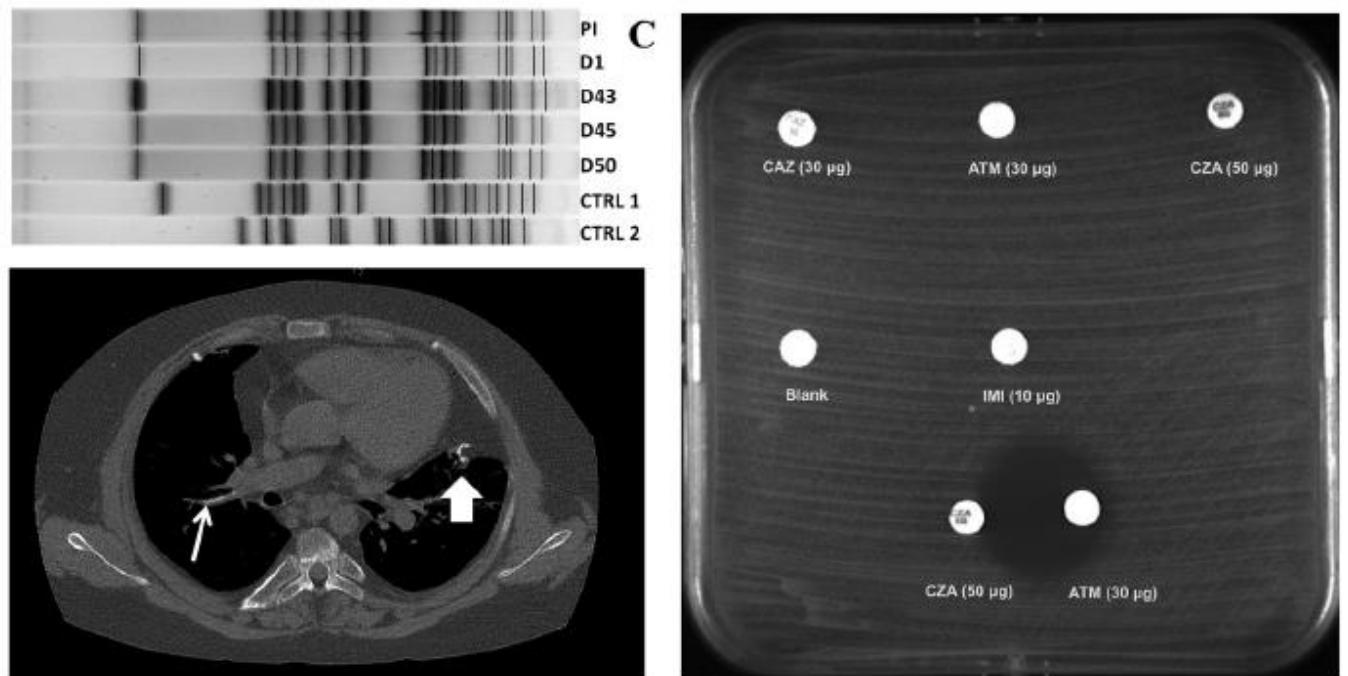
L1



PBP

Successful Treatment of Bloodstream Infection Due to Metallo- β -Lactamase-Producing *Stenotrophomonas maltophilia* in a Renal Transplant Patient

Maria F. Mojica,^{a,b,c} Christopher P. Ouellette,^e Amy Leber,^f M. Brian Becknell,^g Monica I. Ardura,^e Federico Perez,^{a,b,d} Masako Shimamura,^{e,h} Robert A. Bonomo^{a,b,c,d}



Can Ceftazidime-Avibactam and Aztreonam Overcome β -Lactam Resistance Conferred by Metallo- β -Lactamases in *Enterobacteriaceae*?

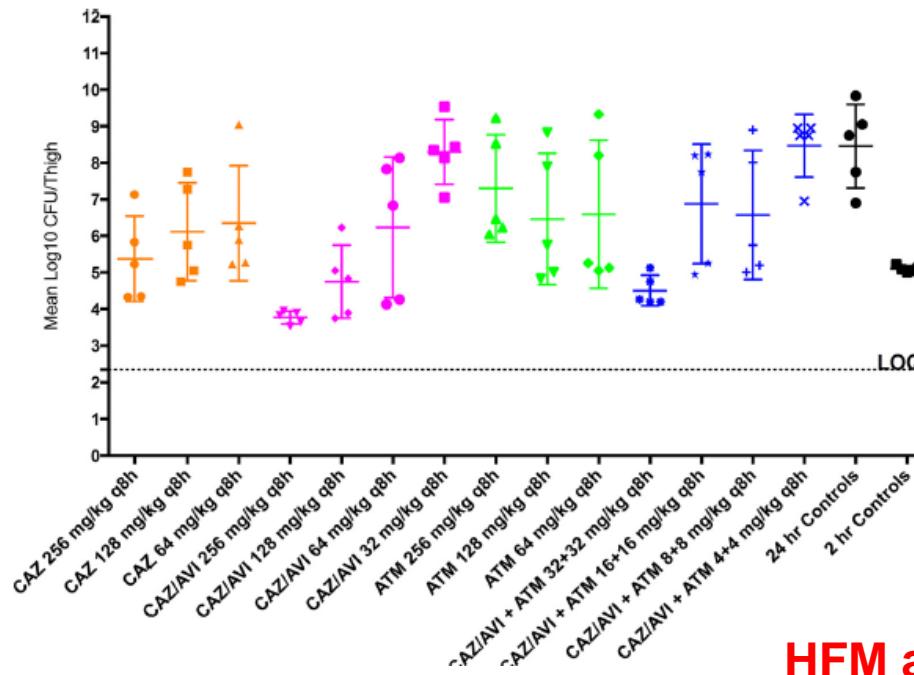
Marshall et al.

NDM producers

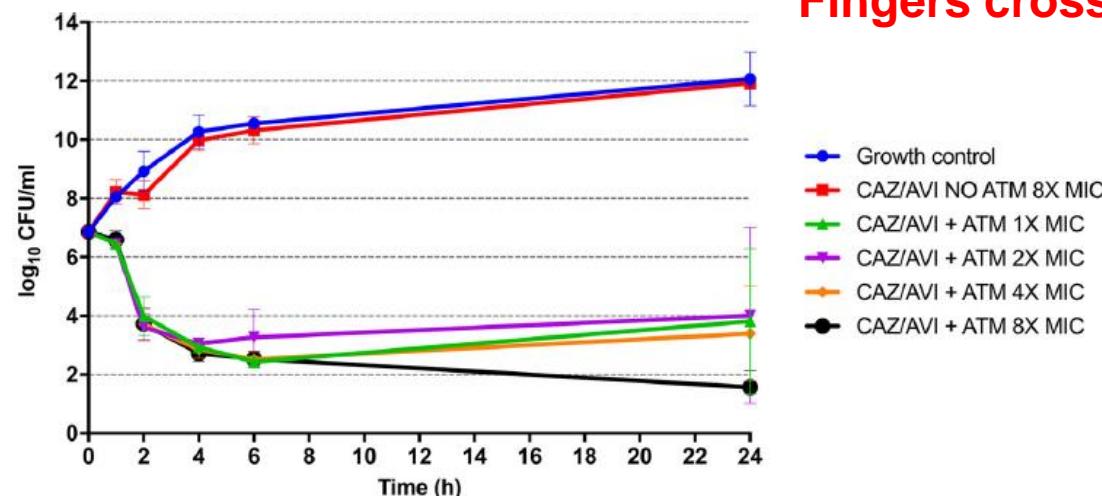
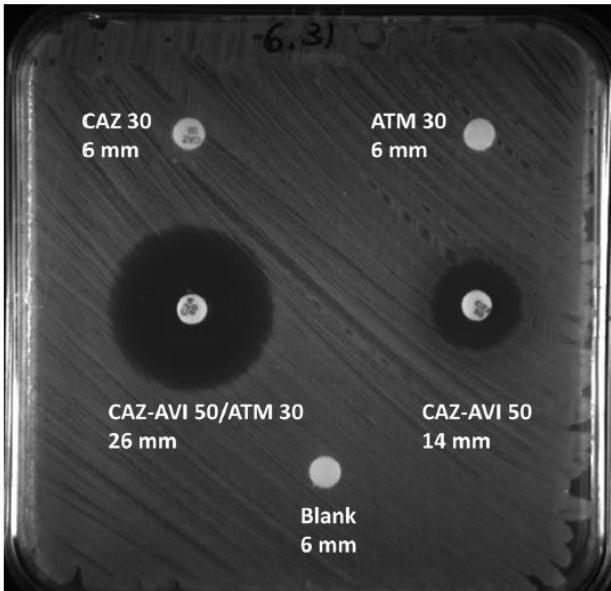
Antimicrobial Agents and Chemotherapy

Steven Marshall,^a Andrea M. Hujer,^{a,b} Laura J. Rojas,^{a,b,c}
Krisztina M. Papp-Wallace,^a Romney M. Humphries,^d Brad Spellberg,^e
Kristine M. Hujer,^{a,b} Emma K. Marshall,^a Susan D. Rudin,^{a,b} Federico Perez,^{a,b}
Brigid M. Wilson,^a Ronald B. Wasserman,^f Linda Chikowski,^g David L. Paterson,^h
Alejandro J. Vilà,ⁱ David van Duin,^j Barry N. Kreiswirth,^k Henry F. Chambers,^l
Vance G. Fowler, Jr.,^m Michael R. Jacobs,ⁿ Mark E. Pulse,^o William J. Weiss,^o
Robert A. Bonomo^{a,b,c,p}

At 2 h $\geq 4 \log_{10}$ decrease
in CFU in time-kill assays
(ATM 8 mg/L)
 $\geq 4 \log_{10}$ decrease
in thigh infection (32
mg/kg CZA and 32 mg/kg
ATM)



HFM and Phase 1
clinical trial
Fingers crossed



RELEBACTAM

Major OmpK36 mutations

stepwise emergence of imi-rel R

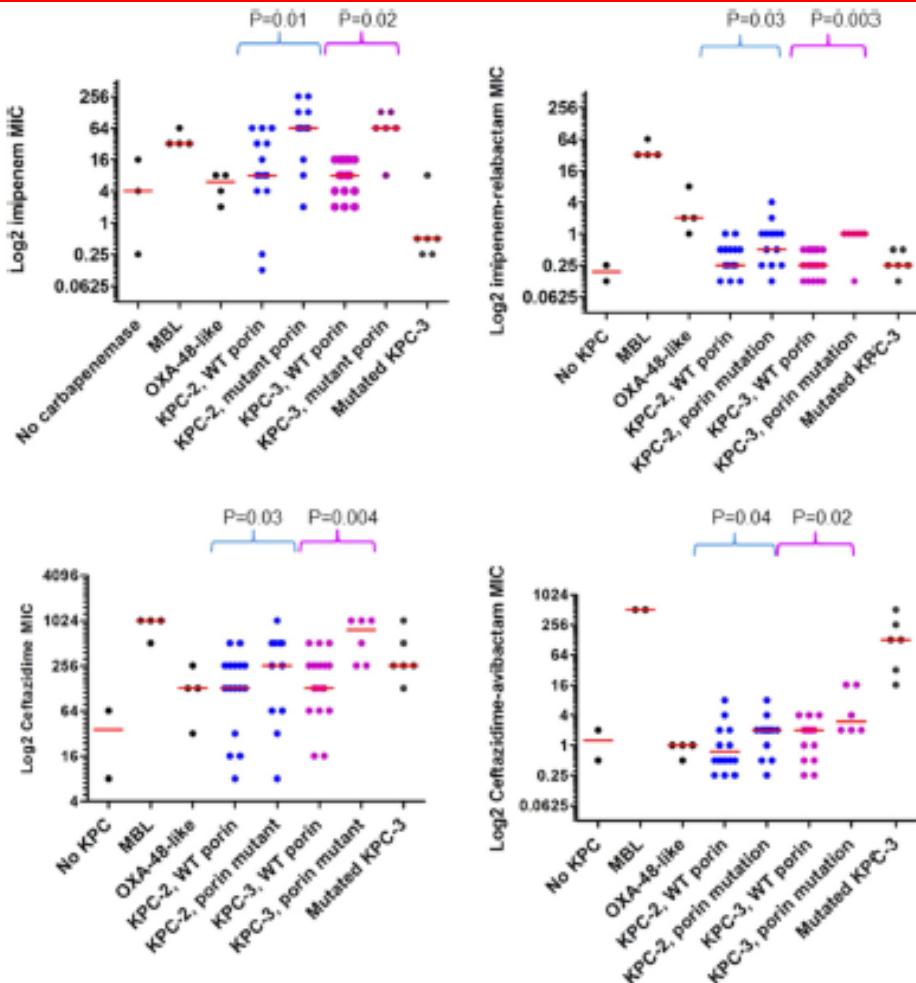


FIG 1 Distributions of imipenem, imipenem-relebactam, ceftazidime, and ceftazidime-avibacam MICs, stratified by type of carbapenemase and porin status. The horizontal red lines represent median MICs.

Identifying Spectra of Activity and Therapeutic Niches for Ceftazidime-Avibactam and Imipenem-Relebactam against Carbapenem-Resistant *Enterobacteriaceae*

© Ghady Haidar,^a Cornelius J. Clancy,^{b,c,d} Liang Chen,^e Palash Samanta,^a Ryan K. Shields,^{a,b,c} Barry N. Kreiswirth,^a M. Hong Nguyen^{a,b,c}

1. Major OmpK36 porin mutations were independently associated with higher imi/rel MICs ($P < 0.0001$) and showed a trend toward independent association with higher taz avi MICs ($P = 0.07$).

2. The presence of variant KPC-3 was associated with taz avi resistance ($P < 0.0001$). In conclusion, imi-rel and taz-avi had overlapping spectra of activity and niches. and taz-avi resistance.

Inactivation of the *Pseudomonas*-Derived Cephalosporinase-3 (PDC-3) by Relebactam

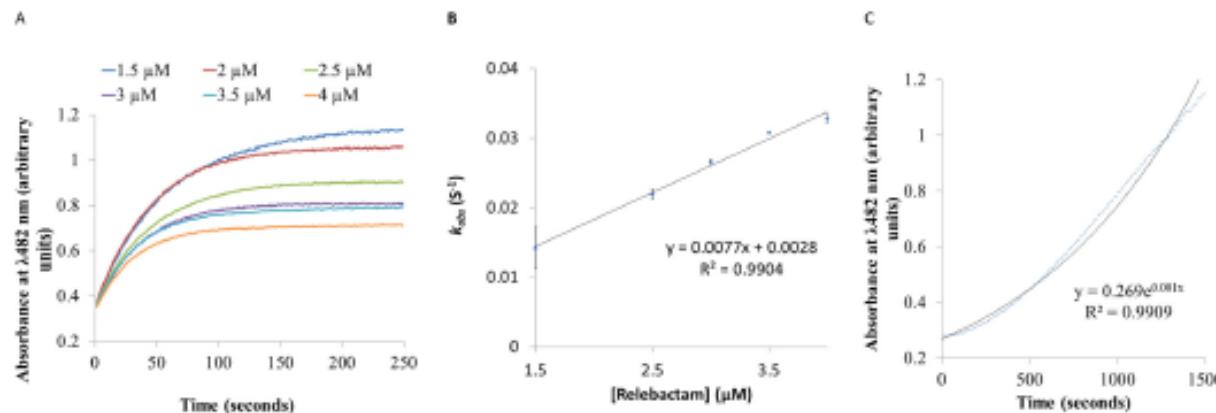
Melissa D. Barnes,^{a,b} Christopher R. Bethel,^a Jim Alsop,^b Scott A. Becka,^a Joseph D. Rutter,^a Krisztina M. Papp-Wallace,^{a,b,e,f} Robert A. Bonomo^{a,b,c,d,e,f,g,i}

TABLE 2 Steady-state kinetic parameters of PDC-3 with relebactam, compared to avibactam

Kinetic parameter	Relebactam	Avibactam ^a
K_{app} (μM)	3.4 ± 0.4	2.5 ± 0.3
k_2/K ($\text{M}^{-1} \text{s}^{-1}$)	$4.1 \times 10^4 \pm 0.5 \times 10^4$	$2.9 \times 10^4 \pm 2.9 \times 10^4$
k_{off} (s^{-1})	$9.5 \times 10^{-4} \pm 0.5 \times 10^{-4}$	$8.0 \times 10^{-4} \pm 0.8 \times 10^{-4}$
Half-life (min)	12.2 ± 0.6	14.4 ± 1.4
K_d (nM)	23 ± 3	28 ± 3
k_{cat}/k_{inact}	1	ND ^b

^aWinkler et al. (14).

^bND, not determined.

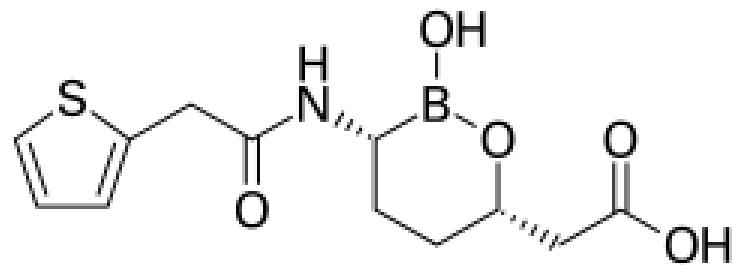


MICs are lower because imipenem is a more potent partner

Pivotal Phase 3 Study of Merck's Investigational Beta-Lactamase Inhibitor Relebactam in Combination with Imipenem/Cilastatin Demonstrated Favorable Overall Response Against Certain Imipenem-Non-Susceptible Bacterial Infections

- RESTORE-IMI 1 study @ 28th ECCMID 2018 (Madrid) 4/21-24.
- Multicenter, randomized, DB, comparator-controlled trial : IMI/REL vs COL+IMI in pts with imi-non-susceptible bacterial infxs.
- Patients with HABP/VABP, cIAI, or cUTI caused by one or more imi-non-susceptible (but Colistin- and IMI/REL susceptible) pathogens, were randomized 2:1 to receive IMI/REL or COL+IMI in a double-blind fashion.
- Study duration was 5-21 days for cUTI and cIAI; 7-21 days for HABP/VABP.
- Favorable overall response was comparable for the IMI/REL (71.4%; n=15) and COL+IMI (70.0%; n=7) treatment arms. **Favorable clinical response at Day 28 was higher in the IMI/REL arm (71.4%; n=15) compared to the COL+IMI (40.0%; n=4) arm, and 28-day all-cause mortality was lower in the IMI/REL arm (9.5%; n=2) vs. COL + IMI (30.0%; n=3), respectively.**
- adverse events occurred **in 16.1% of patients (n=31) in the IMI/REL arm vs. 31.3% of patients (n=16) in the COL+IMI arm. Treatment-emergent nephrotoxicity was lower with IMI-REL (10%; 3/29)**

VABORBACTAM



Are these “druggable”....Jump forward to novel boranates in development

Journal of
**Medicinal
Chemistry**

Drug Annotation

pubs.acs.org/jmc

Discovery of a Cyclic Boronic Acid β -Lactamase Inhibitor (RPX7009) with Utility vs Class A Serine Carbapenemases

Scott J. Hecker,^{*†} K. Raja Reddy,[†] Maxim Totrov,[‡] Gavin C. Hirst,[†] Olga Lomovskaya,[†] David C. Griffith,[†] Paula King,[†] Ruslan Tsivkovski,[†] Dongxu Sun,[†] Mojgan Sabet,[†] Ziad Tarazi,[†] Matthew C. Clifton,[§] Kateri Atkins,[§] Amy Raymond,[§] Kristy T. Potts,[§] Jan Abendroth,[§] Serge H. Boyer,[†] Jeffrey S. Loutit,[†] Elizabeth E. Morgan,[†] Stephanie Durso,[†] and Michael N. Dudley[†]

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[‡]Molsoft L.L.C., 11199 Sorrento Valley Road, San Diego, California 92121, United States

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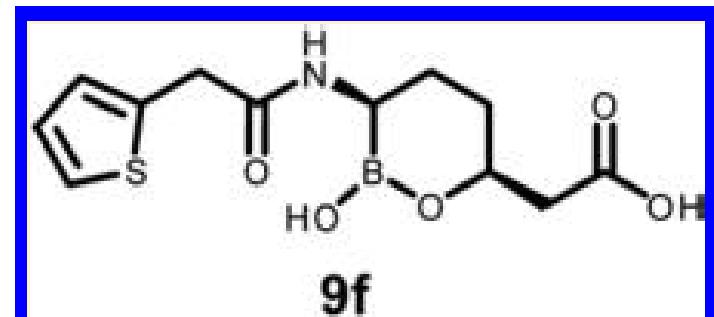
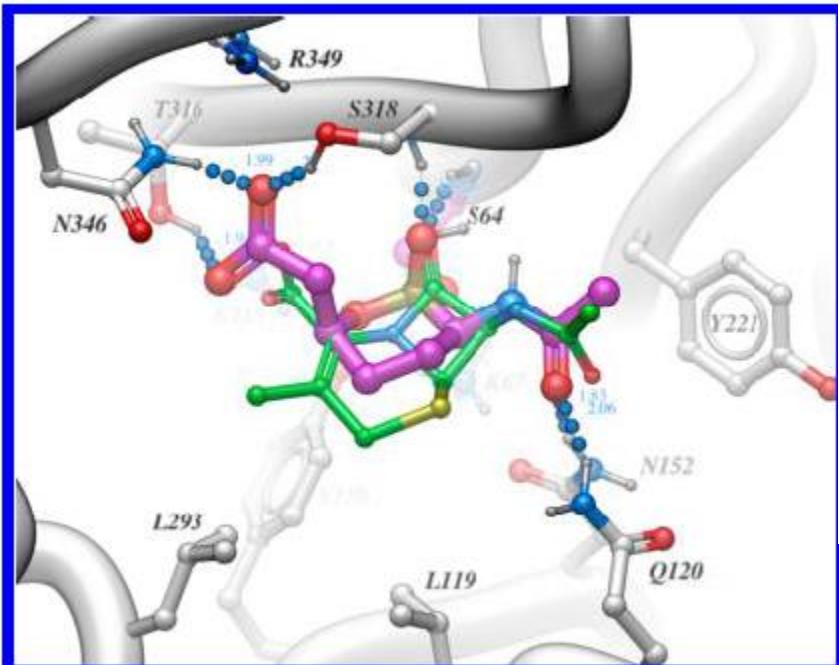


Table 2. Inhibition of Nitrocefin Degradation (K_i , μM) by Compound 9f

enzyme	class	9f	clavulanic acid	tazobactam
KPC-2	A	0.069	41.2	1.6
CTX-M-15	A	0.044	0.027	0.001
SHV-12	A	0.029	≤ 0.039	0.0004
TEM-10	A	0.110	0.020	0.005
P99	C	0.053	1106	1.10
CMY-2	C	0.099	845	0.71



Ki= 0.07 vs KPC; Ki= 0.03 vs SHV-12

Ceftazidime/avibactam, Meropenem/vaborbactam or both?

Clinical and formulary considerations

Authors and affiliations

CID

Jason M. Pogue, PharmD^{1,2}, Robert A. Bonomo, MD^{3,4,5}, Keith S. Kaye, MD, MPH⁶

JAMA | Original Investigation

Effect of Meropenem-Vaborbactam vs Piperacillin-Tazobactam on Clinical Cure or Improvement and Microbial Eradication in Complicated Urinary Tract Infection The TANGO I Randomized Clinical Trial

Keith S. Kaye, MD, MPH; Tanaya Bhowmick, MD; Symeon Metallidis, MD; Susan C. Bleasdale, MD; Olexiy S. Sagan, MD; Viktor Stus, MD, PhD; Jose Vazquez, MD; Valerii Zaitsev, PhD; Mohamed Bidair, MD; Erik Chorvat, MD; Petru Octavian Dragoescu, MD; Elena Fedosiuk, MD; Juan P. Horcajada, MD, PhD; Claudia Murta, MD; Yaroslav Sarychev, MD; Ventsislav Stoev, MD; Elizabeth Morgan, BS; Karen Fusaro, BS; David Griffith, BS; Olga Lomovskaya, PhD; Elizabeth L. Alexander, MD; Jeffery Loutit, MBChB; Michael N. Dudley, PharmD; Evangelos J. Giamarellos-Bourboulis, MD, PhD

- **Phase 3, MC, MN, RCT (TANGO I) conducted 11/ 2014 to 4/ 2016**
- **Patients (≥ 18 years) with c UTI, stratified by infection type and geographic region**

TANGO I

- Eligible patients were randomized 1:1 to receive meropenem-vaborbactam (2g/2g over 3 hours; n = 274) or piperacillin-tazobactam (4g/0.5g over 30 minutes; n = 276) every 8 hours.
- After 15 or more doses, patients could be switched to oral levofloxacin if they met prespecified criteria for improvement, to complete 10 days of total treatment.

TANGO I

- For the FDA primary end point, overall success occurred in 189 of 192 (98.4%) with meropenem-vaborbactam vs 171 of 182 (94.0%) with piperacillin-tazobactam (difference, 4.5% [95% CI, 0.7% to 9.1%]; $P < .001$ for noninferiority).

How do you translate these studies to CREs? MDROs?

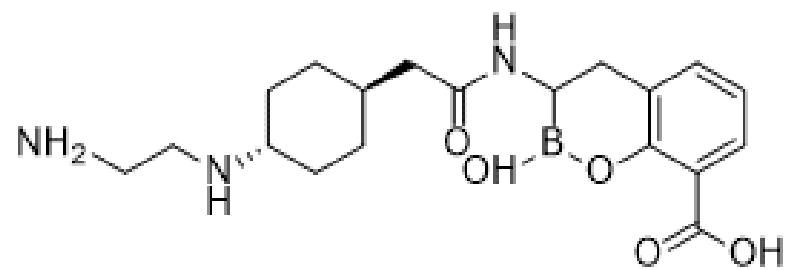
Table 1: In vitro activity of ceftazidime/avibactam and meropenem/vaborbactam against problematic Gram-negative pathogens

TAZ AVI ≠ Mero Vabor

Organism	Resistance present	Ceftazidime/avibactam	Meropenem/vaborbactam
Enterobacteriaceae			
	ESBL	+++	+++
	AmpC	+++	+++
	KPC	+++	+++
	MBL	-	*
	OXA-48 like	+++	*
<i>A. baumannii</i>			
	Carbapenem-resistant	-	-
<i>P. aeruginosa</i>			
	Carbapenem-resistant	++	-
	Pan beta-lactam resistant	+	-
<i>S. maltophilia</i>			
	Ceftazidime-resistant	-	-

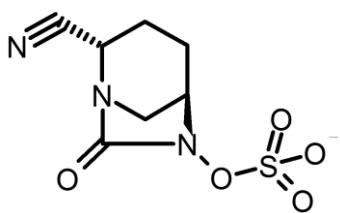
ESBL = extended spectrum beta lactamase, KPC = Klebsiella pneumonia carbapenemase, MBL = metallo-beta-lactamase, Pan beta-lactam resistant = resistant to traditional anti-pseudomonal beta lactams (cefepime, ceftazidime, meropenem, imipenem, and piperacillin/tazobactam)

+++ = activity >90%, would expect in vitro activity; ++ = activity 60-90%, high likelihood of in vitro activity, but ensure susceptibility prior to using; + = activity 30-60%, lower likelihood of susceptibility; - minimal to no activity, should not be considered an option; * might have activity, but driven by meropenem, vaborbactam does not add anything in this setting.

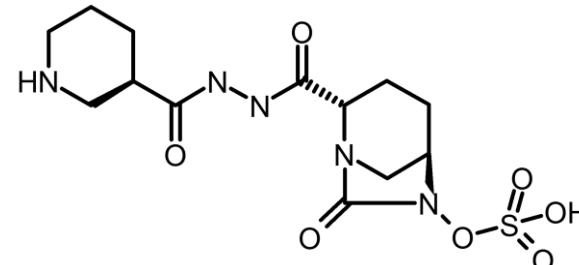


VNRX 5133

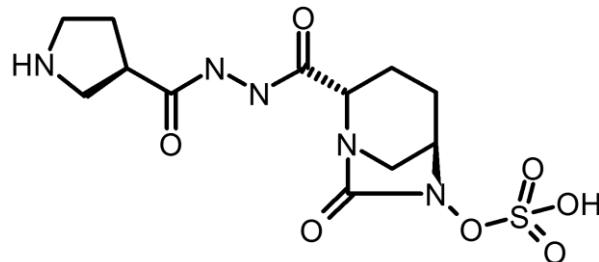
Novel DBOs: forging new paths



WCK 4234



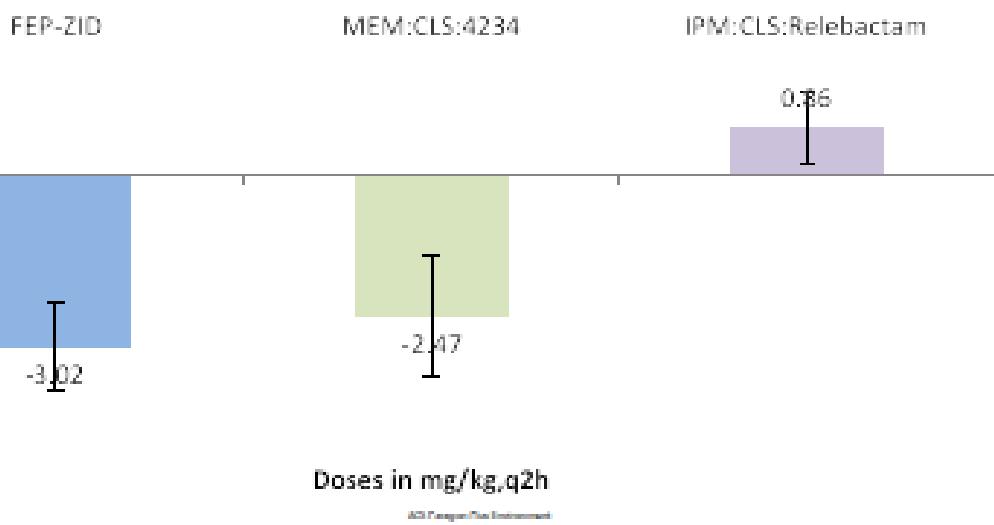
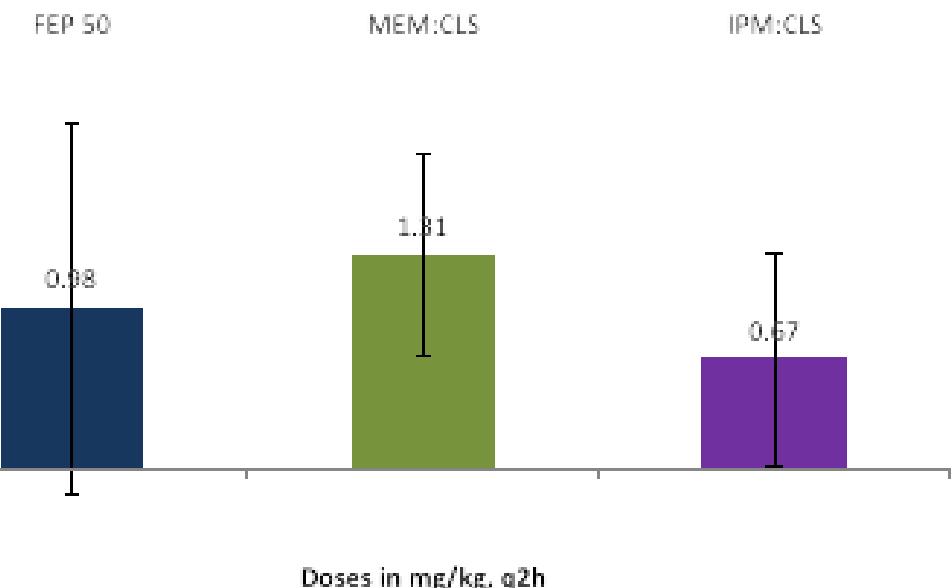
WCK 5107



WCK 5153

1. BLEs represent a new antimicrobial class and work by providing complimentary PBP inhibition to a partner β -lactam.
2. By targeting two different PBPs, BLEs act synergistically (*P. Aeruginosa* and *A. baumannii*)
3. Operate independently of BLIs.

A murine neutropenic lung infection model using *A. baumannii* SL06 carrying *blaOXA-23* and *blaOXA-51*. The graphs represent the change in CFU/lung after different antibiotic treatments administered as q2h.).



EXT2514+ Sulbactam

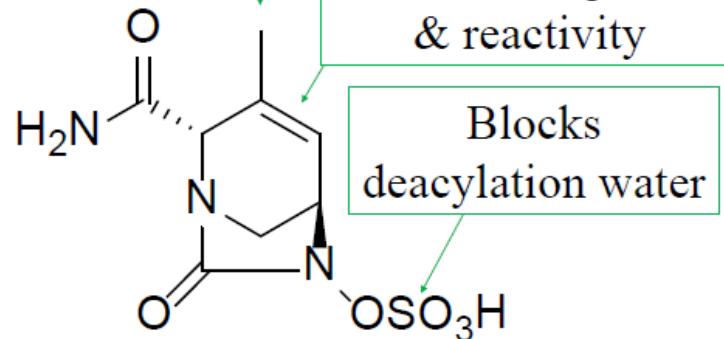
Form the basis
of a phase III trial



CDC Threat Report, 2013¹

Structure of ETX2514

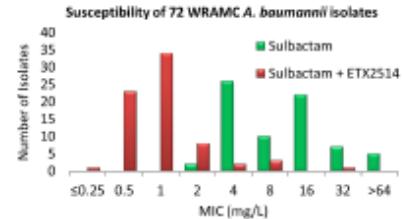
Hydrophobic interactions



Increases ring strain
& reactivity

Blocks
deacylation water

ETX2514 overcomes resistance of the WRAMC isolates to sulbactam



	Sulbactam	Sulbactam-ETX2514
<i>E. coli</i> pBC SK (-) bla _{ADC-7}	32	<0.03
<i>A. baumannii</i> MIC ₅₀	8	1
<i>A. baumannii</i> MIC ₉₀	32	2

Figure 4: MICs of the WRAMC *A. baumannii* isolates Medical Center Isolates were performed on agar broth dilution according to the Clinical and Laboratory Standards Institute⁷.

ETX2514 effectively inhibits ADC-7 and OXA-58 β -lactamases

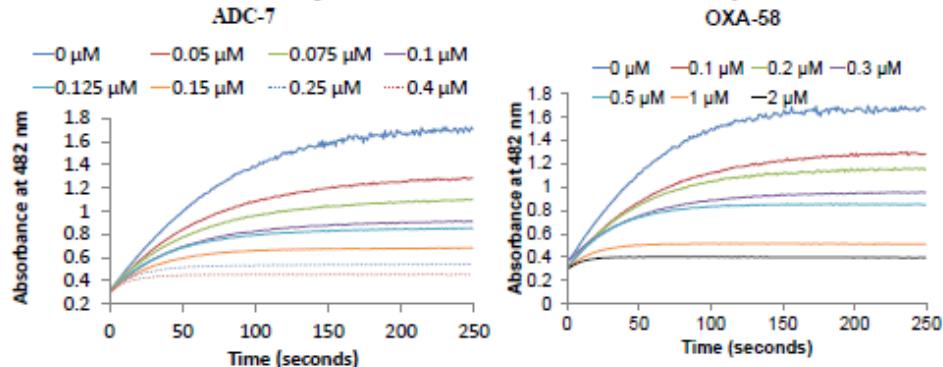
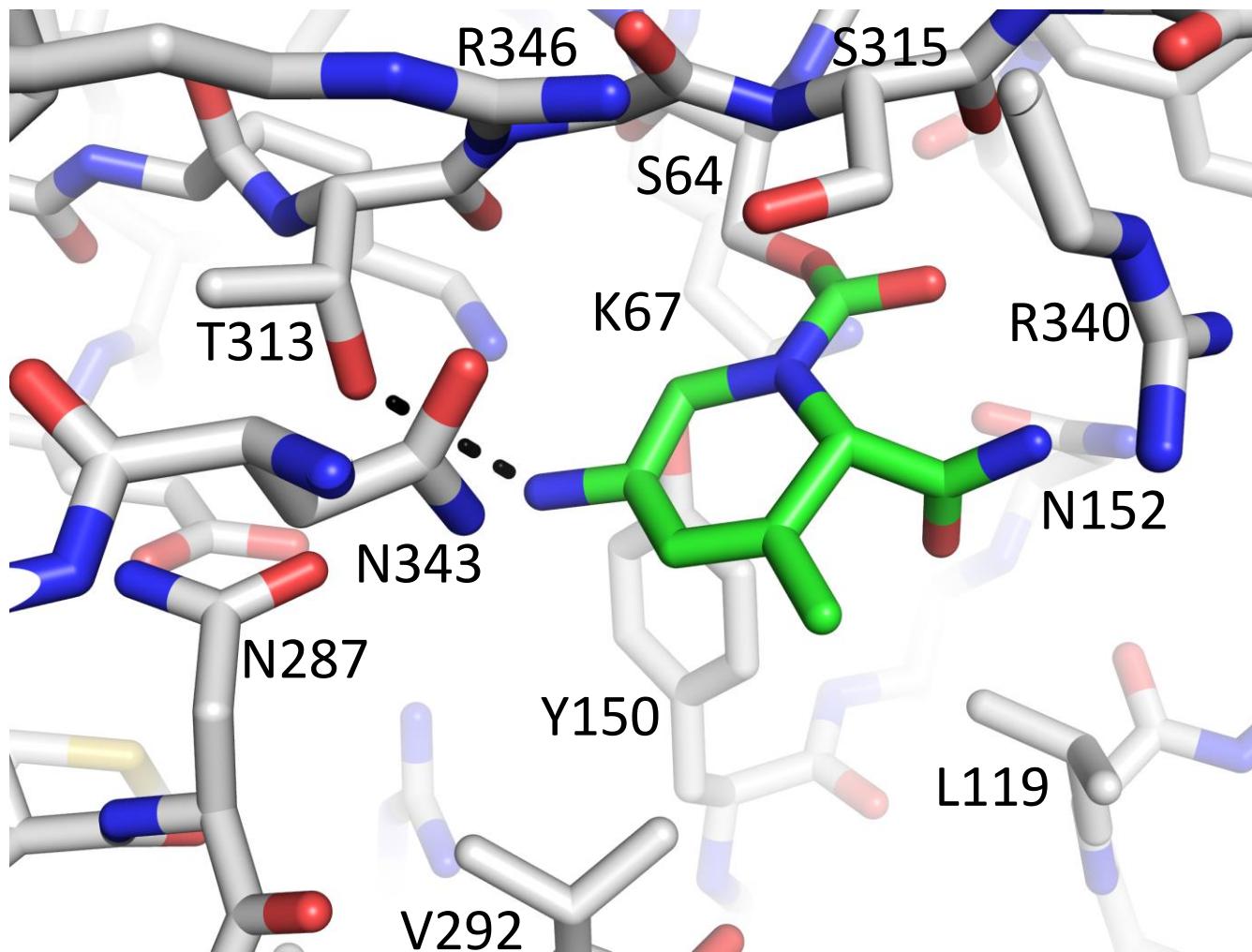


Figure 5: β -lactamases were mixed with increasing concentrations of ETX2514 using 100 μM NCF as a reporter substrate.

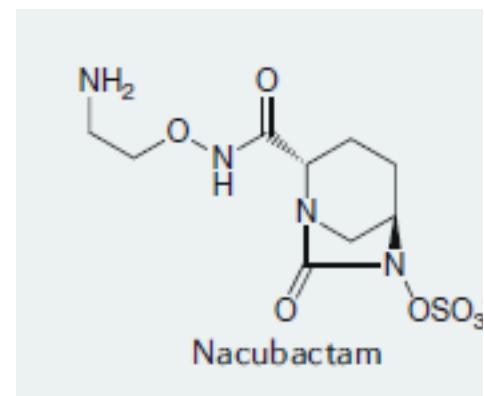
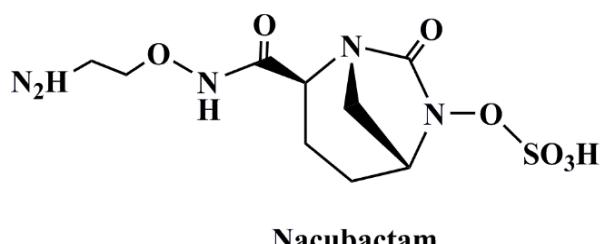
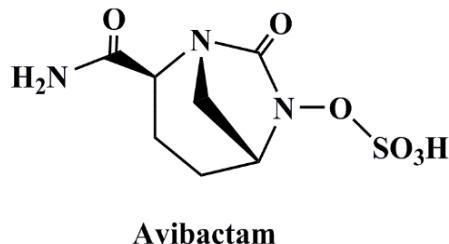
Desulfated fragment of ETX2514 bound to ADC-7



ETX2514 fragment makes a covalent bond with S70, a hydrogen bond with T313, and has its carbonyl oxygen located outside the oxyanion hole. 2.2 Angstroms

Nacubactam

- Nacubactam, formerly **RG6080** and **OP0595**, is a member of the growing class of bridged diazabicyclooctane (DBO) β -lactamase inhibitors that differs from avibactam with the **addition of an aminoethoxy group to the carbomyl side chain present on avibactam**



Nacubactam MOA

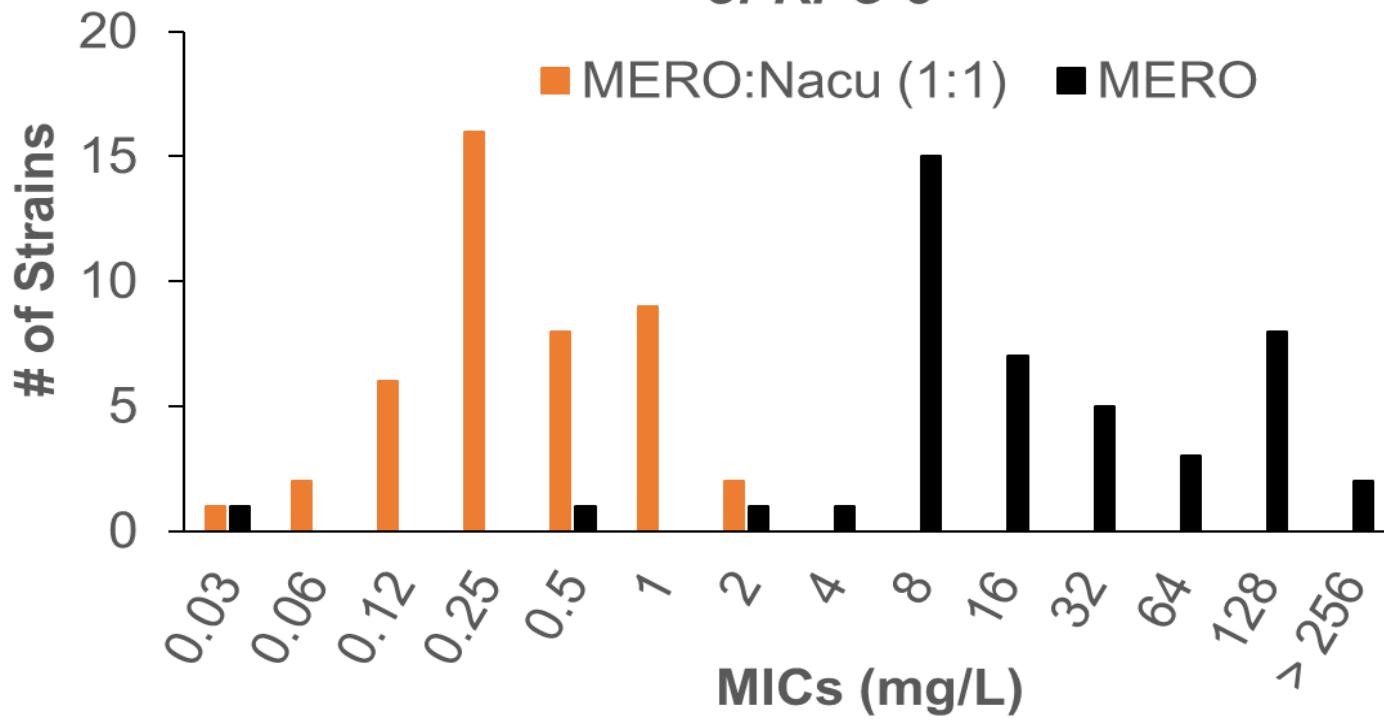
This **aminoethoxy group to the carbomyl side chain** is likely responsible for the significant intrinsic antibiotic activity of nacubactam alone.

Analogous to ETX2514, and the WCK 5153 and zidebactam DBOs, nacubactam inhibits *Escherichia coli* penicillin binding protein 2 (**PBP2**) .

Moreover, nacubactam was reported to act synergistically as a β -lactam **enhancer**, like mecillinam and the WCK 5153 and zidebactam DBOs, when combined with other β -lactams.

This “**enhancer**” effect is the result of these combinations possessing the ability to target multiple PBPs.

K. pneumoniae clinical strains harboring KPC-2 or KPC-3



MICs of 44 *K. pneumoniae* clinical isolates containing KPC-2 or KPC-3 β -lactamases tested against meropenem (MERO) and MERO combined with nacubactam (Nacu) at a 1:1 ratio.

SAR based MICs

- We hypothesized that the structure and catalytic determinants (i.e., amino acids 73, 104, 105, 130, 164, 166, 170, 179, 220, 234, 235, 237, 240, and 276) that mediate inactivation of class A β -lactamases by β -lactamase inhibitors, such as oxapenems, sulfones, and DBOs would also play a role in the inactivation mechanism of nacubactam.
- Isogenic panel of *E. coli* producing KPC-2 variants with single amino acid substitutions at critical active site residues (73, 104, 105, 130, 164, 166, 170, 179, 220, 234, 235, 237, 240, and 276)
- KPC-2 **K234R** variant was found to demonstrate increased meropenem-nacubactam minimum inhibitory concentration (MIC) compared to KPC-2, yet did not possess an increase in meropenem MIC.

KPC-2 Substitution	Nacubactam	Meropenem Nacubactam (1:1)	Meropenem	Ertapenem	Aztreonam	Aztreonam Avibactam (4 mg/L)	Ceftazidime Avibactam (4 mg/L)	Cefepime	Piperacillin Tazobactam (4 mg/L)	Colistin	Amikacin	Tigecycline	Levofloxacin	Fosfomycin
	N	N+M	M											

pBC SK vector														
KPC-2 (wt)	1	0.12	1	1	> 32	0.12	0.25	2	128	≤ 0.12	1	0.12	0.015	2
H274Y (KPC-3)	2	0.06	0.5	0.5	32	0.06	0.25	2	64	≤ 0.12	1	0.12	0.015	2
P104R V240G (KPC-4)	2	0.12	0.25	0.5	> 32	0.12	2	4	64	≤ 0.12	2	0.12	0.015	2
P104R (KPC-5)	2	0.06	0.12	0.12	> 32	0.12	0.25	1	32	≤ 0.12	2	0.12	0.015	2
V240G (KPC-6)	2	0.06	0.25	0.12	> 32	0.12	0.5	2	64	≤ 0.12	2	0.12	0.015	2
M49I H274Y (KPC-7)	2	0.06	0.25	0.25	32	0.06	0.25	2	64	≤ 0.12	2	0.12	0.015	2
V240G H274Y (KPC-8)	2	0.12	0.5	0.5	> 32	0.12	1	4	128	≤ 0.12	1	0.12	0.015	2
K73A	2	0.015	0.01	0.008	0.25	0.12	0.5	0.06	2	0.025	2	0.12	0.03	2
K73R	2	0.03	0.01	0.008	0.12	0.12	0.25	0.15	4	≤ 0.12	2	0.12	0.015	2
P104A	2	0.06	0.5	0.5	> 32	0.06	0.25	2	64	0.25	2	0.5	0.015	4
P104K	2	0.25	1	1	> 32	0.12	0.5	4	128	0.5	2	0.5	0.015	4
W105A	1	0.06	0.06	0.12	32	0.06	0.12	0.25	32	0.25	1	0.12	0.015	2
S130G	1	0.03	0.01	0.008	0.25	0.06	0.12	0.06	> 128	0.25	2	0.12	0.015	2
S130T	2	0.06	0.03	0.06	0.12	0.06	0.25	0.12	4	≤ 0.12	1	0.12	0.015	2
E166A	2	0.03	0.06	0.015	0.25	0.06	0.25	0.12	2	0.25	2	0.12	0.015	2
E166Y	2	0.03	0.03	0.06	2	0.06	0.5	1	1	≤ 0.12	2	0.06	0.015	2
N170A	4	0.06	0.03	0.06	1	0.12	2	2	64	≤ 0.12	2	0.12	0.015	2
N170P	2	0.06	0.03	0.25	4	0.12	4	4	4	0.25	2	0.12	0.015	2
R220K	1	0.06	0.06	0.12	2	0.06	0.12	0.12	2	0.25	2	0.12	0.015	2

Taz avi

N N+M M



R220M	1	0.03	0.03	0.15	0.25	0.06	0.12	0.12	32	0.25	1	0.06	0.015	2
T235A	2	0.03	0.03	0.015	1	0.06	0.12	0.06	32	0.25	2	0.12	0.015	2
T235S	2	0.03	0.12	0.016	0.5	0.06	0.12	0.06	4	0.25	4	0.5	0.015	2
T237A	1	0.03	0.06	0.25	16	0.06	0.12	0.06	32	0.25	2	0.12	0.015	2
T237S	1	0.06	0.5	1	> 32	0.06	0.25	1	64	0.25	1	0.12	0.015	2
V240G	2	0.25	4	8	> 32	0.25	2	16	> 128	0.25	2	0.12	0.015	2
V240K	2	0.25	2	4	> 32	0.12	0.5	4	> 128	0.25	2	0.06	0.015	2
E276A	1	0.12	0.5	1	32	0.06	0.25	1	64	0.25	2	0.12	0.015	2
E276N	2	0.06	0.25	0.5	16	0.12	0.12	0.5	64	0.25	2	0.12	0.015	2

pBR322-catI- vector

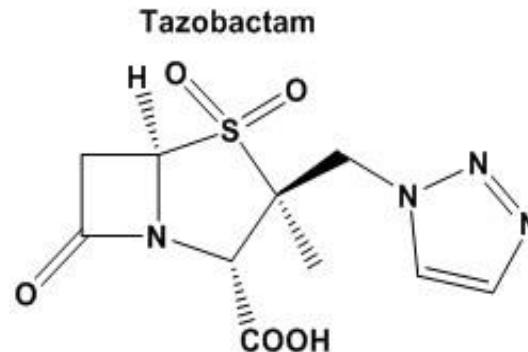
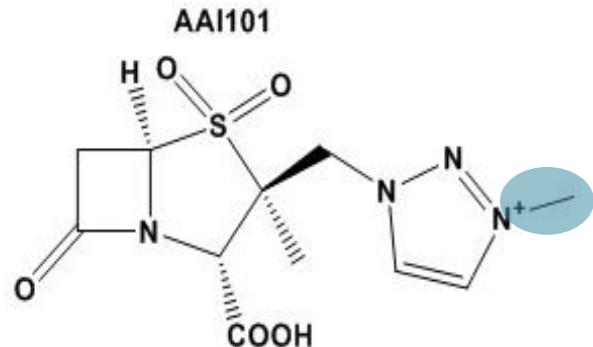
wt	2	0.25	8	32	> 32	0.25	1	16	> 128	0.25	0.5	0.12	0.03	2
R164A	2	0.25	0.5	2	> 32	0.12	4	8	> 128	0.25	0.5	0.06	0.015	2
R164H	2	0.25	2	4	> 32	0.12	1	> 32	> 128	0.25	≤ 0.25	0.06	0.015	2
R164P	2	0.06	0.03	0.12	0.25	0.12	8	0.5	2	≤ 0.12	0.5	0.06	0.015	2
R164S	2	0.12	1	2	> 32	0.06	2	8	> 128	0.25	≤ 0.25	0.06	0.015	2
D179A	2	0.06	0.03	0.12	1	0.12	8	2	2	0.25	0.5	0.06	0.015	2
D179C	1	0.06	0.06	0.5	8	0.12	8	1	2	≤ 0.12	0.5	0.12	0.015	2
D179E	1	0.03	0.01	0.03	1	0.12	2	0.12	2	≤ 0.12	1	0.12	0.015	4
D179F	2	0.06	0.06	0.12	4	0.12	8	4	2	≤ 0.12	≤ 0.25	0.06	0.015	2
D179G	4	0.12	0.12	0.25	8	0.12	8	8	8	≤ 0.12	1	0.06	0.015	2
D179H	2	0.06	0.03	0.12	0.25	0.12	8	1	1	≤ 0.12	0.5	0.06	0.015	2
D179I	1	0.015	0.01	0.15	0.25	0.12	8	0.5	2	0.25	2	0.12	0.015	2
D179K	2	0.03	0.03	0.03	0.12	0.12	4	0.25	4	≤ 0.12	≤ 0.25	0.06	0.015	2
D179L	2	0.06	0.03	0.03	2	0.12	8	2	2	≤ 0.12	≤ 0.25	0.06	0.015	2
D179M	2	0.06	0.06	0.12	1	0.12	8	4	2	≤ 0.12	0.5	0.06	0.015	2
D179N	2	0.25	2	4	> 32	0.12	2	8	> 128	≤ 0.12	0.5	0.06	0.015	2
D179P	1	0.03	0.03	0.06	1	0.12	8	1	1	≤ 0.12	≤ 0.25	0.12	0.015	2

Taz avi

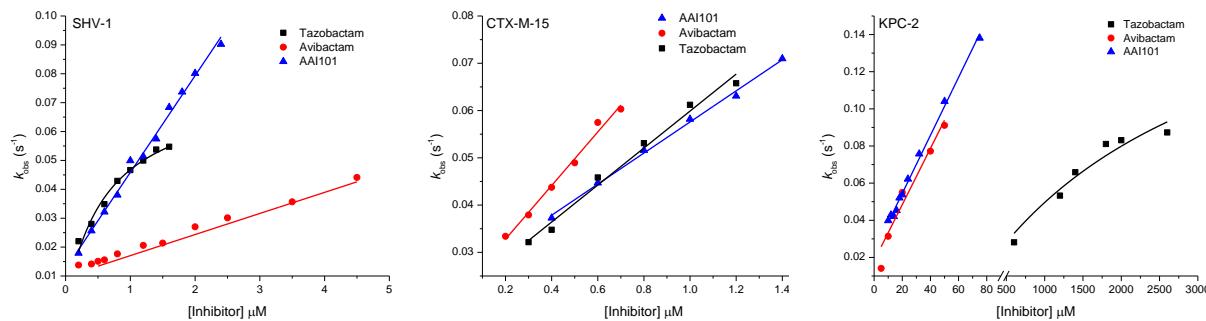


N N+M M

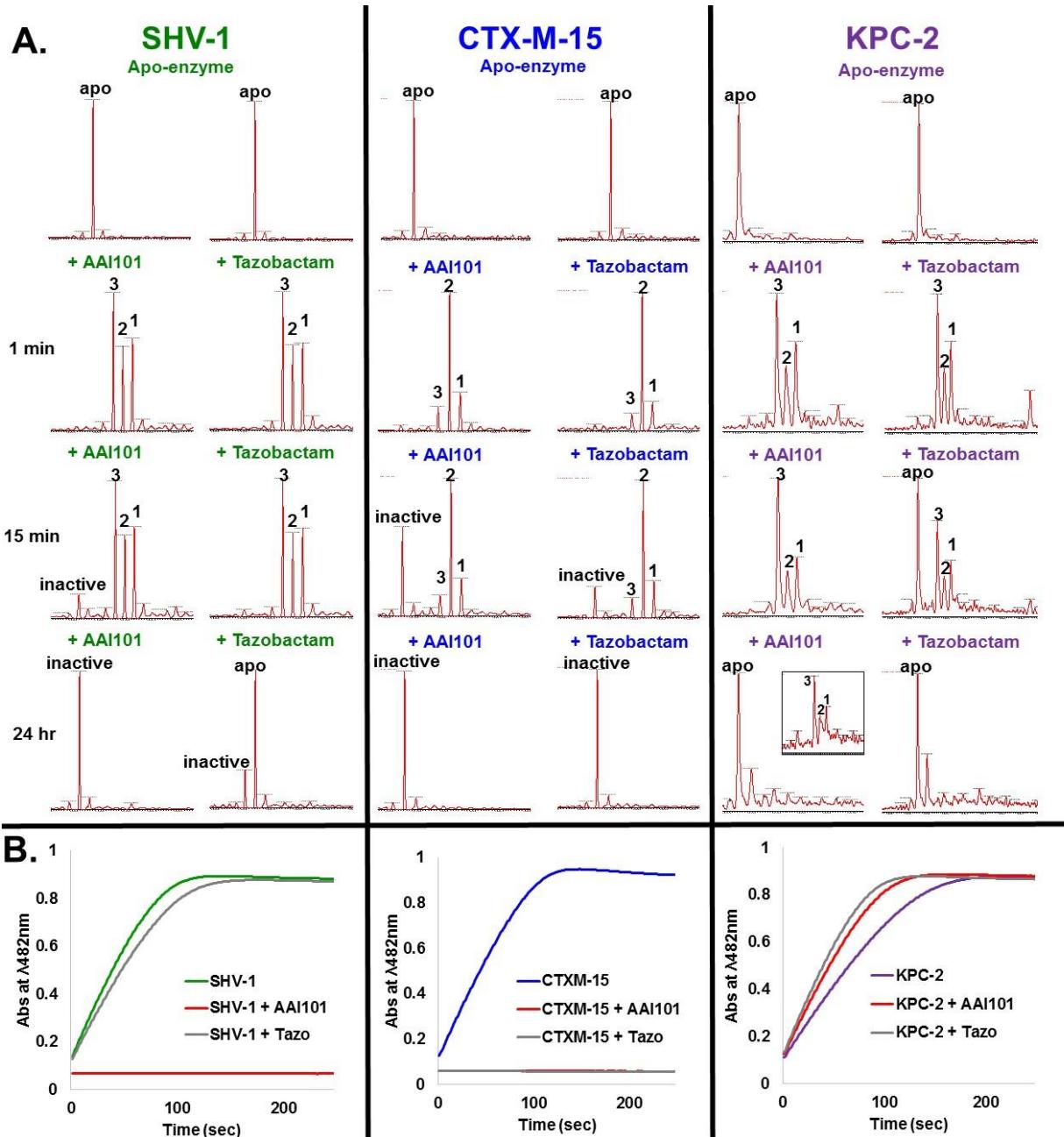
D179Q	2	0.06	0.03	0.12	0.25	0.12	4	0.25	1	≤ 0.12	0.5	0.06	0.015	2
D179R	1	0.06	0.03	0.06	0.12	0.12	4	0.12	1	≤ 0.12	0.5	0.06	0.015	2
D179S	1	0.03	0.015	0.03	1	0.06	1	0.5	2	0.25	1	0.12	0.015	2
D179T	1	0.06	0.03	0.12	1	0.12	8	1	2	≤ 0.12	≤ 0.25	0.06	0.015	2
D179V	1	0.06	0.03	0.12	1	0.12	8	2	1	≤ 0.12	0.5	0.12	0.015	2
D179W	2	0.06	0.06	0.03	4	0.12	8	4	2	≤ 0.12	0.5	0.12	0.015	2
D179Y	2	0.06	0.06	0.25	1	0.12	8	2	2	≤ 0.12	0.5	0.12	0.015	2
R220A	2	0.12	0.12	0.25	1	0.06	0.25	0.5	> 128	0.25	≤ 0.25	0.06	0.015	2
K234A	1	0.015	0.01	0.008	0.12	0.06	0.12	0.03	1	0.25	0.5	0.12	0.015	2
K234R	2	0.5	2	4	32	0.12	0.25	8	> 128	0.25	0.5	0.06	0.015	2

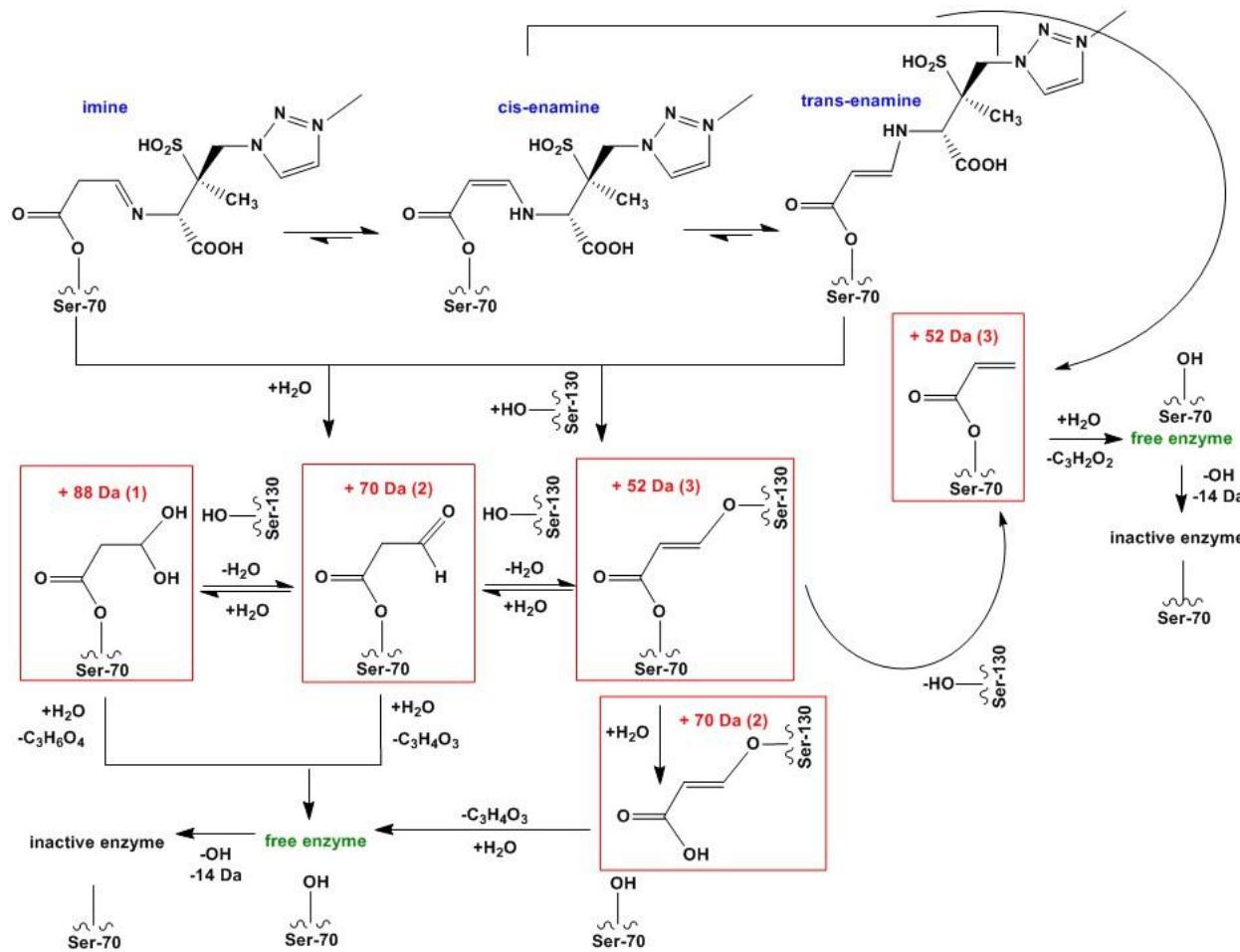


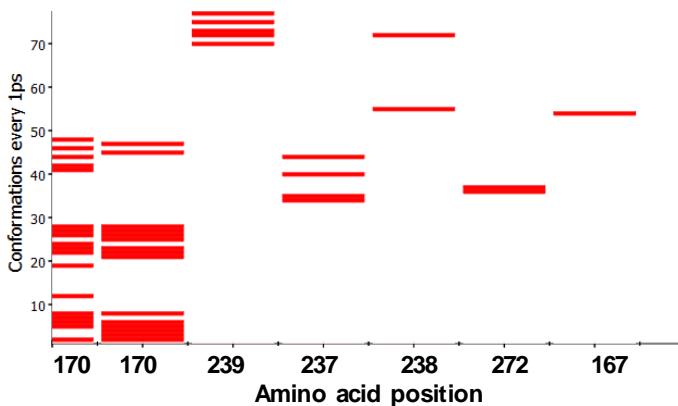
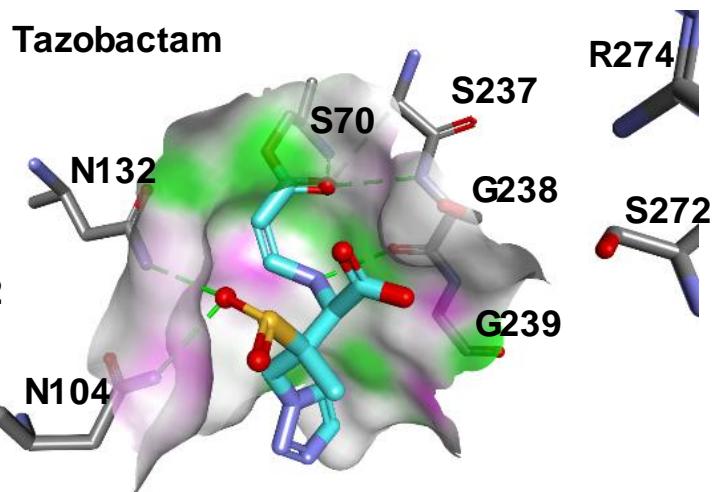
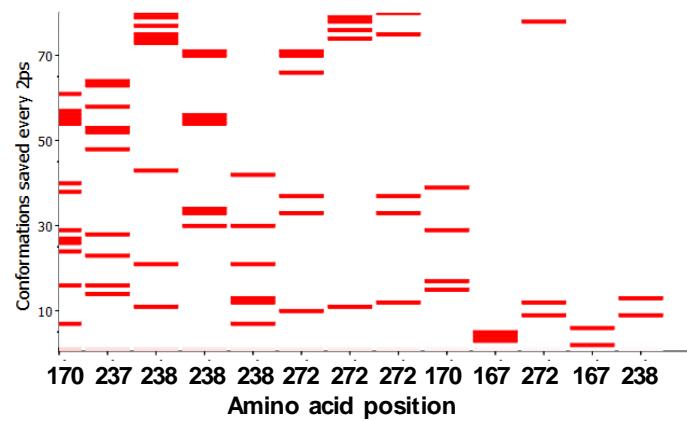
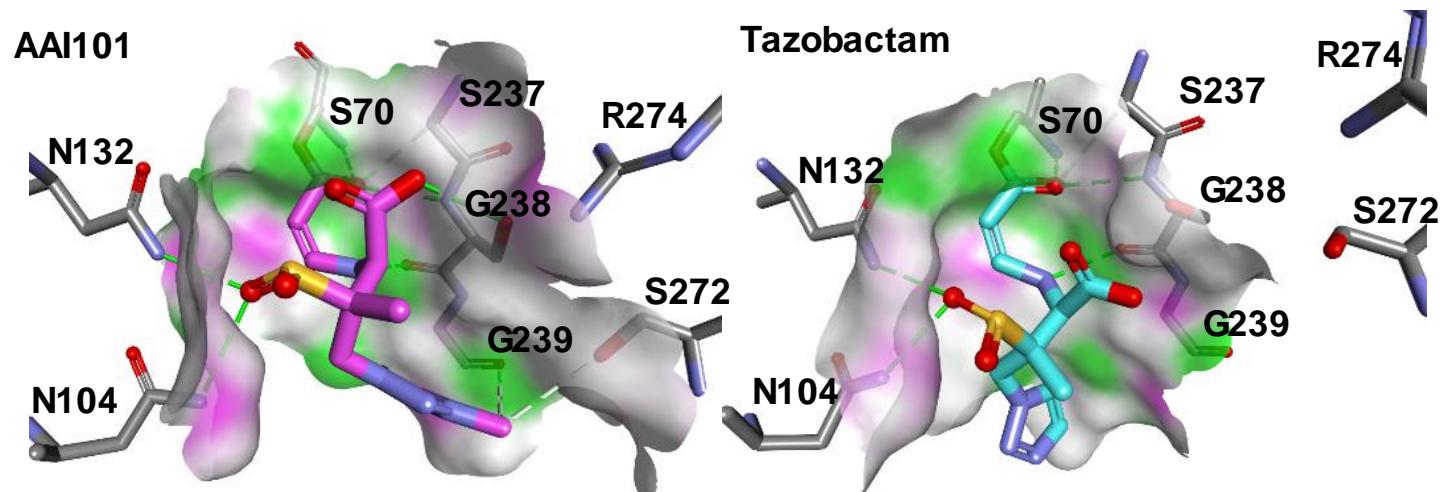
AAI101 possesses a strategically placed methyl group that gives the inhibitor a net neutral charge enhancing bacterial cell penetration.

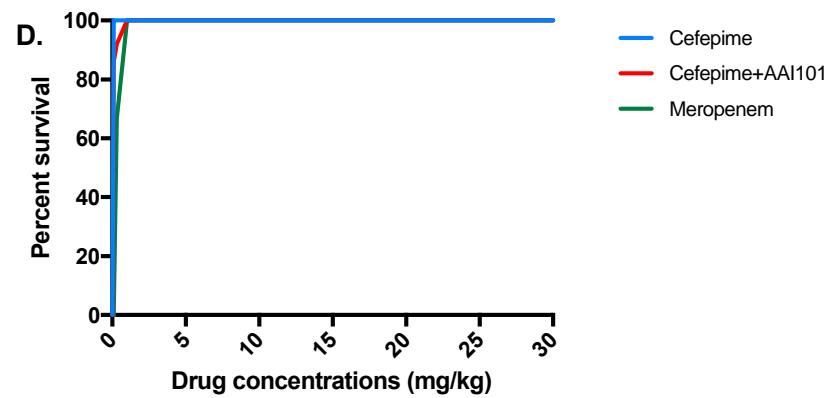
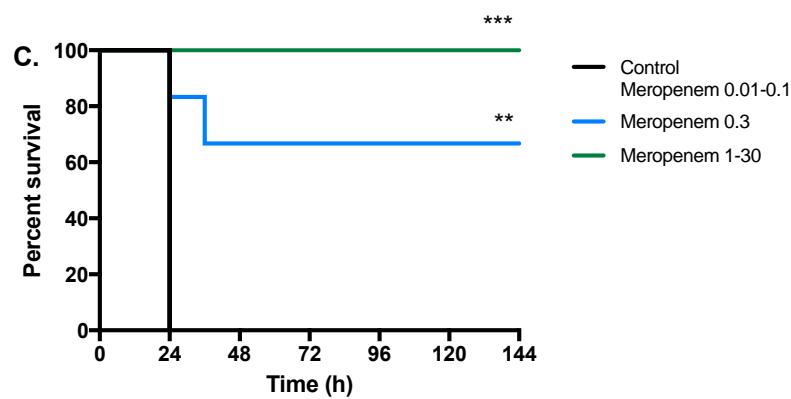
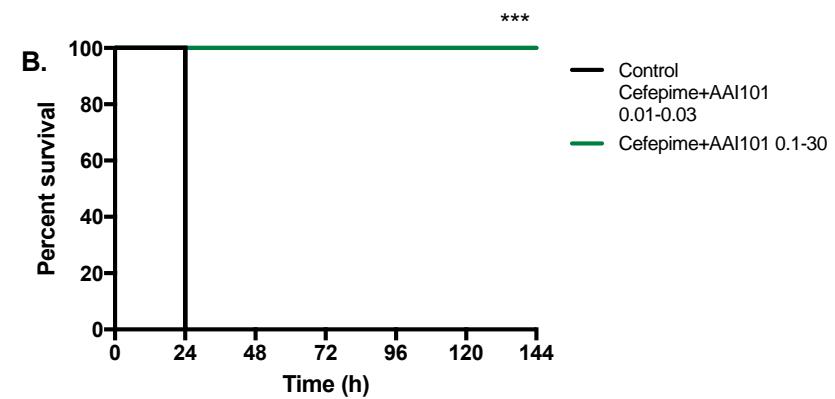
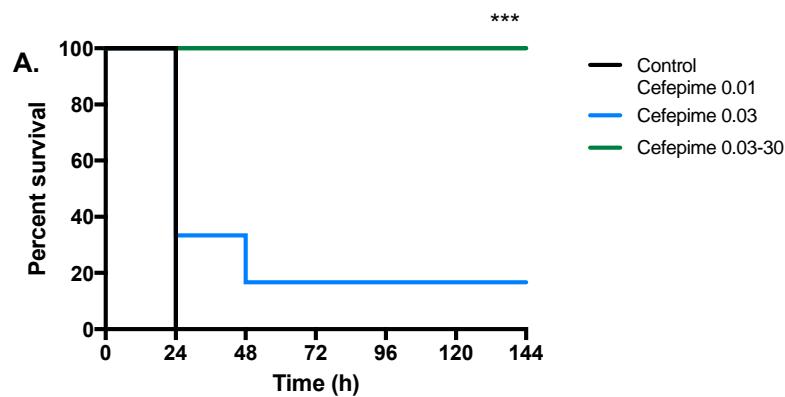


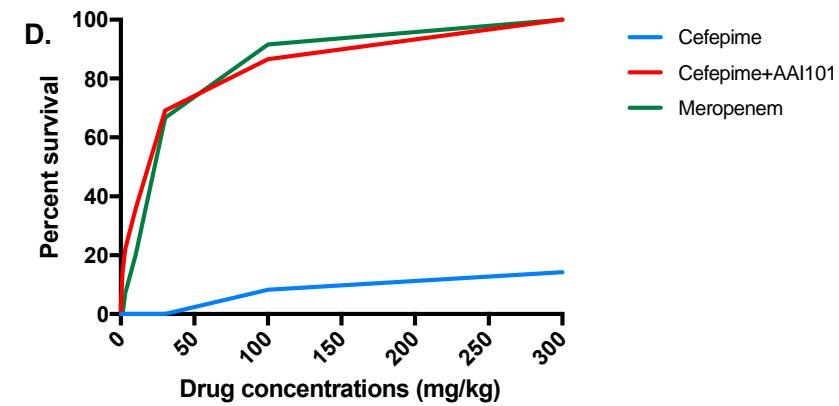
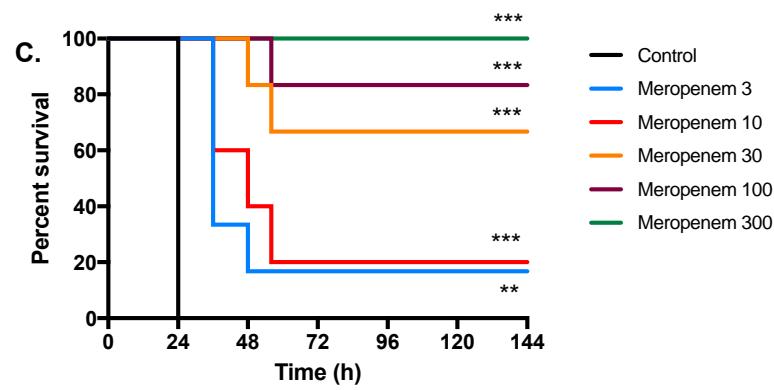
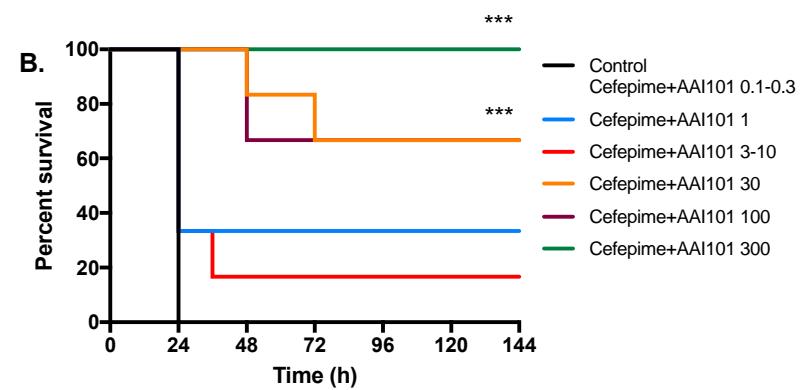
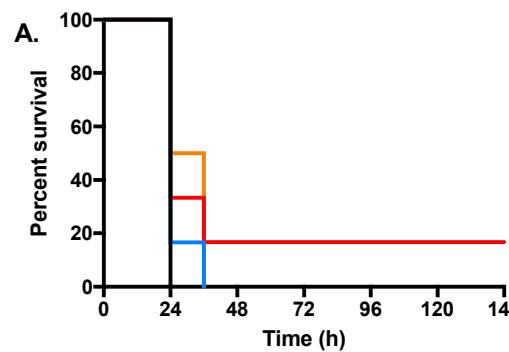
AAI101 possessed a unique mechanism of β -lactamase inhibition compared to tazobactam





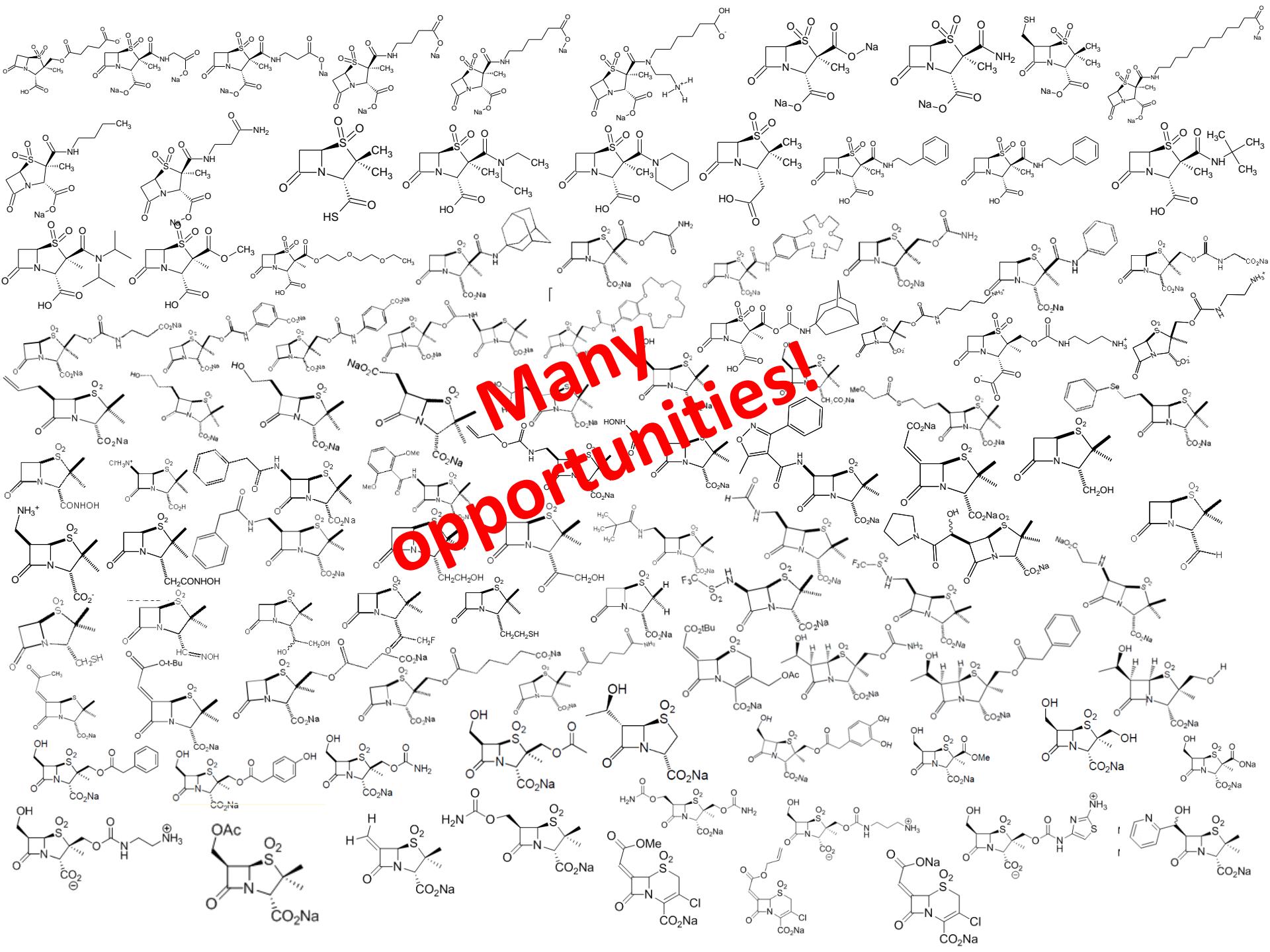






In a mouse septicemia model AAI101 improves the therapeutic efficacy of cefepime *in vivo*.

Many
opportunities!





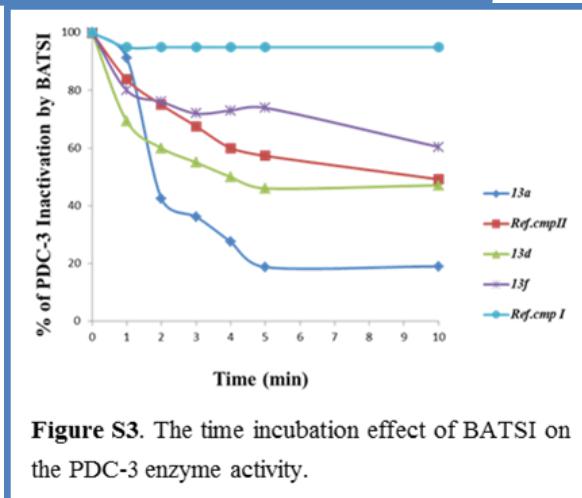
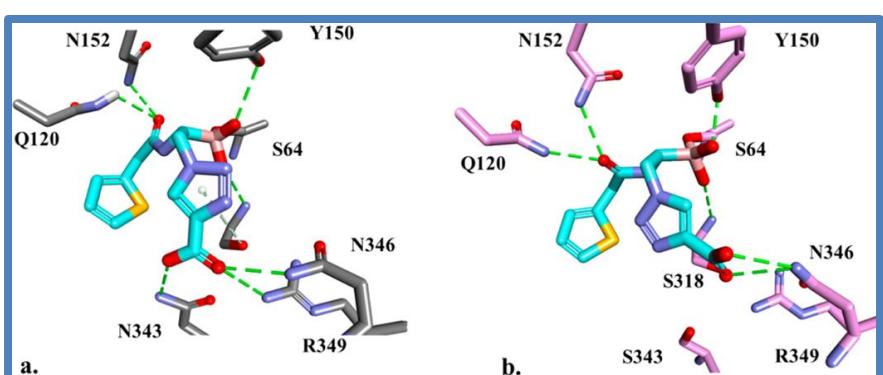
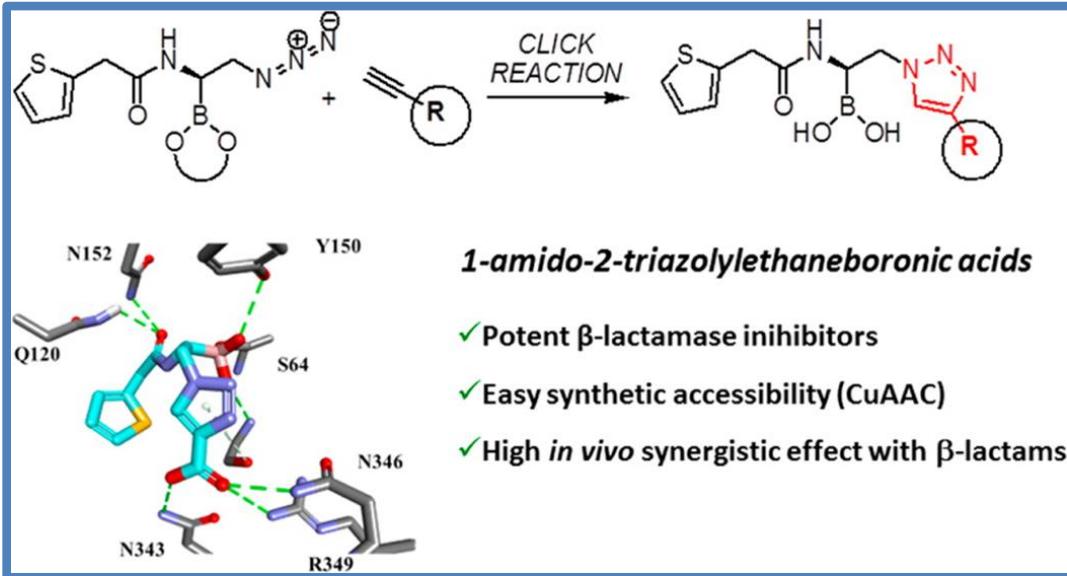
Closing Reflections



Click Chemistry in Lead Optimization of Boronic Acids as β -Lactamase Inhibitors

Emilia Caselli,[†] Chiara Romagnoli,[†] Roza Vahabi,^{†,II} Magdalena A. Taracila,[§] Robert A. Bonomo,^{‡,§} and Fabio Prati^{*,†}

Exploring
new
“chemical
Space”



Can one inhibit *Acinetobacter* (ADC)?

BIOCHEMISTRY
including biophysical chemistry & molecular biology

2014 Dec; 53: 7670-9

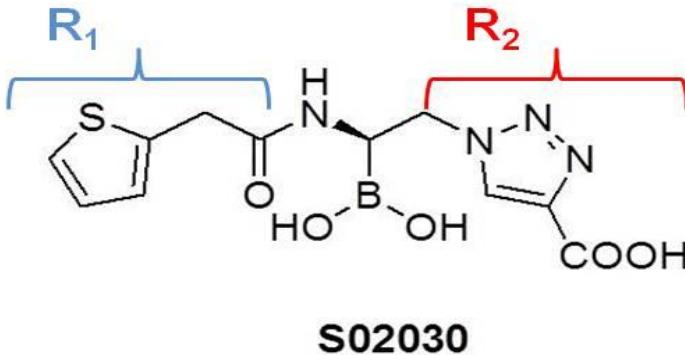
Article

pubs.acs.org/biochemistry

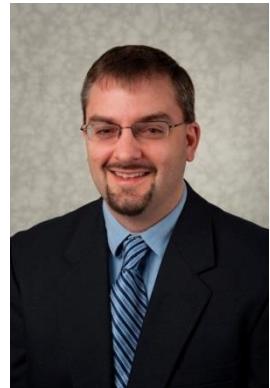
1 Biochemical and Structural Analysis of Inhibitors Targeting the 2 ADC-7 Cephalosporinase of *Acinetobacter baumannii*

3 Rachel A. Powers,[†] Hollister C. Swanson,[†] Magdalena A. Taracila,^{§,||} Nicholas W. Florek,[†]

4 Chiara Romagnoli,[‡] Emilia Caselli,[‡] Fabio Prati,[‡] Robert A. Bonomo,^{*,§,||} and Bradley J. Wallar^{*,†}



Specific modifications (sulfonyl, benzyl, triazole moieties) to optimize structure activity relationships (SAR)



Next steps

Toxicology, determine pharmacokinetic parameters in the mouse (Cmax, AUC, Vd, Cl), etc
Need \$ € £ ¥

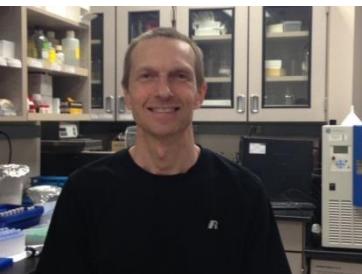
**Does S02030 work against other
β-lactamases?**

SHV?

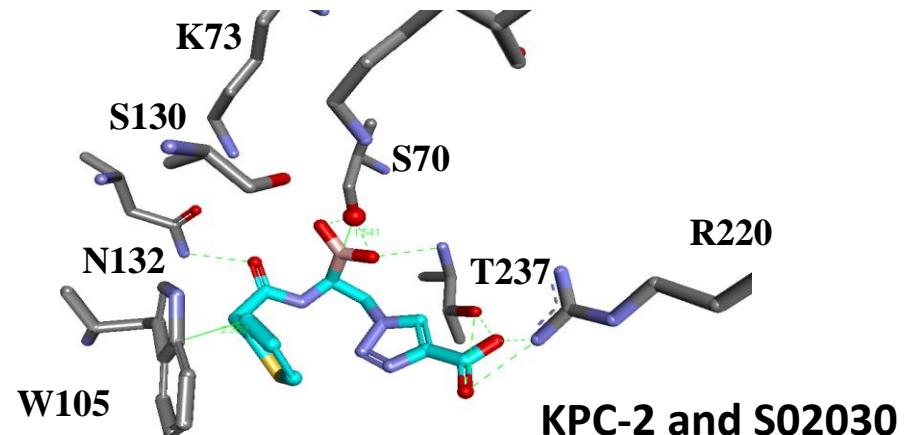
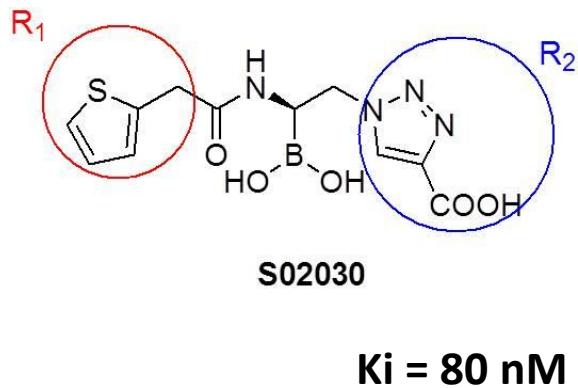
TEM?

CTX-M?

KPC?



Also works vs. *Klebsiella* KPC



Agar dilution MICs

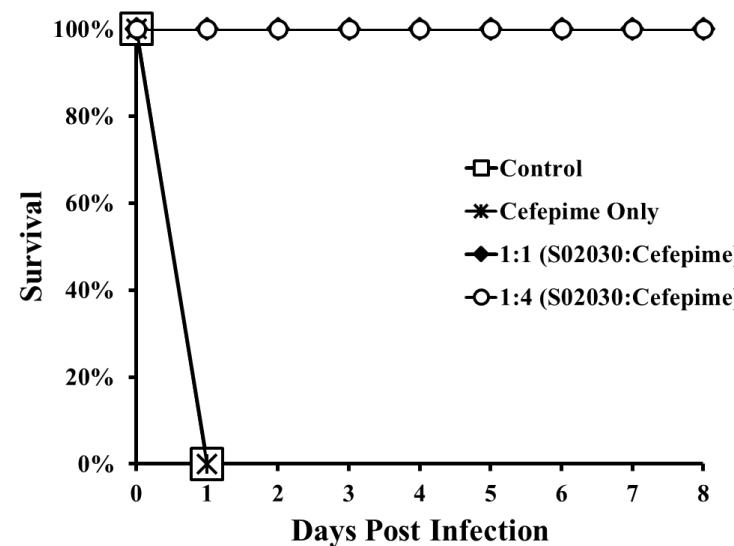
Strain	β -lactamase	Agar dilution MICs					
		FEP	S02030		S02030		S02030
		FEP	ERT	ERT	CAZ	CAZ	
DH10B	none	0.06	0.06	0.06	0.06	1	0.25
<i>E.coli</i> DH10B PBR322	KPC-2	16	0.25	8	0.06	64	4
<i>K. pneumoniae</i> (<i>Kp</i>)	KPC-2	16	0.125	16	0.5	64	2
<i>Kp</i> VA375	KPC	16	0.5	8	0.06	64	8
<i>Kp</i> VA388	KPC	8	0.125	4	0.06	64	2
<i>Kp</i> ST258	KPC-2	64	>8	>16	>1	>64	>8
<i>Kp</i> ST258	KPC-3	32	0.5	16	1	64	2
<i>Kp</i> ST17	KPC	64	0.125	8	0.06	32	2
<i>E. coli</i> PR247 (<i>Ec</i>)	KPC	16	0.25	16	0.25	64	2
<i>E. coli</i> pLTCF 1	KPC	8	0.125	0.125	0.06	8	0.5

Does it matter....

Kinetics show
that S02030 is
slow to associate
and to
disassociate
Behave as slow
binding
inhibitors

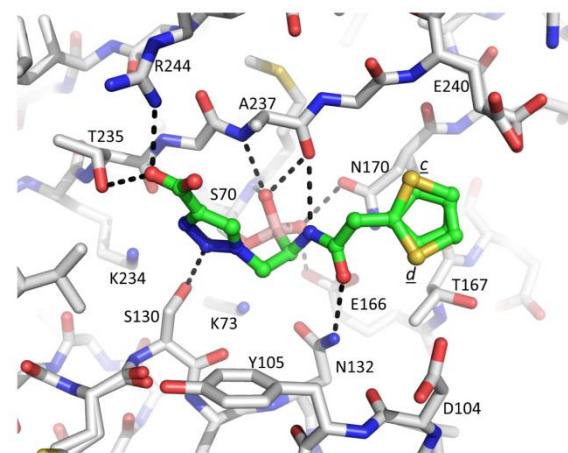
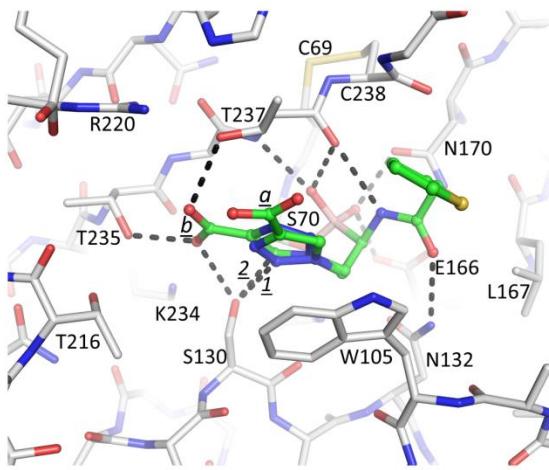
$K_i = 0.08 \pm 0.002 \text{ uM}$
for KPC-2 and $0.13 \pm 0.002 \text{ uM}$ for SHV

Animal model- S02030-Cefepime combination
in collaboration with Dr. Brad Spellberg Keck School of Medicine
at University of Southern California, Los Angeles, California



Inoculum	
Aim =	1.9E+8 CFUs
Actual =	2.1E+8 CFUs
Actual/Aim =	111%

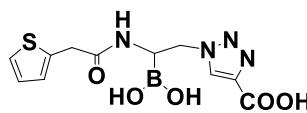
KPC-2



SHV-1

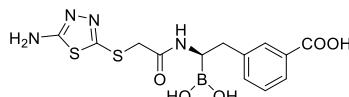


From this series we further identified 6 candidates similar with S02030 to inhibit class A (KPC-2) and C β -lactamases (ADC-7). The best one seems to be MB-076

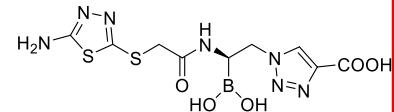


$IC_{50}=50nM$

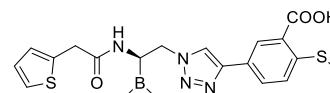
Series I



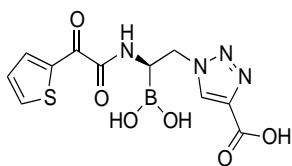
$IC_{50}=70nM$



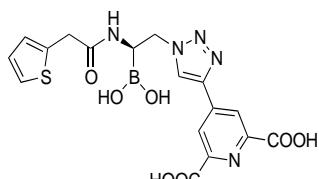
$IC_{50}=100nM$



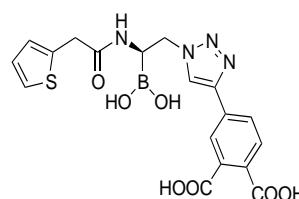
$IC_{50}=500nM$



$IC_{50}=1\mu M$



$IC_{50}=2-5\mu M$



$IC_{50}=2\mu M$

* IC_{50} are determined for ADC-7 enzyme.

The compounds did not work for metallo lactamases at 20 μM

COMP	FEP + BATSI KPC-2	IMI+ BATSI NDM-1	CAZ+ BATSI ADC-7	ADC-7 IC_{50} [μM]
Abt. Partner [mm]	10	8	12	
1 s17067	17	10	18	≈ 20
2 s17068	14	8	12	≈ 20
3 s17069	15	10	17	≈ 20
4 s17070	21	12	20	≈ 5
5 s17071	16	10	20	≈ 5
6 s17075	15	7	12	>20
7 s17076	16	8	12	≈ 5
8 s17077	14	10	22	≈ 5
9 s17078	10	7	20	≈ 5
10 s17079	20	11	20	≈ 5
11 s17080	18	10	12	>20
12 s17081	15	8	12	>20
13 s17082	12	9	12	>20
14 s17083	20	12	22	≈ 1
15 ME_088	18	8	20	≈ 5
16 ME_089	16	7	12	>20
17 ME_090	21	8	12	>20
18 ME_091	19	10	12	>20
19 ME_092	18	12	17	>20
20 ME_093	20	10	12	>20
21 ME_094	15	10	15	>20
22 ME_096	25	8	20	≈ 1
23 ME_097	16	8	16	>20
24 MB054	28	8	27	0.07
25 MB076	25	8	27	0.1
26 S15084	23	8	16	0.5
27 S02030	28	8	26	0.05

Disk assays against class A, class C and metallo β -lactamases were performed.

Disks with 10 μ g of BAI and 10 μ g of partner antibiotic were added and *E.coli*, DH10B β -lactamases strains in pBCSK(-) vector.

The partner antibiotic was cefepime (FEP) for KPC-2, ceftazidime (CAZ) for ADC-7 and imipenem (IMI) for NDM-1.

OBS:

IC_{50} 's were performed with and without 5 min time incubation of BAI and enzyme,

Microbiological data for different strains of class A β -lactamases and selected BATSI – series I

MIC's were performed on M-H agar plates, with 4 μ g/ml BATSI and increasing concentration of cefepime (FEP).				
Strains	FEP	SO2030+FEP	MBO54+FEP	MBO76+FEP
<i>E.coli</i> DH10B	0.25	≤ 0.25	0.25	≤ 0.25
<i>E.coli</i> SHV-1_ATCC BAA-202	8	0.5	1	≤ 0.25
<i>E.coli</i> DH10B SHV-1_pBC SK (-)	2	≤ 0.25	0.5	≤ 0.25
SHV-2_pBCSK	4	≤ 0.25	2	≤ 0.25
<i>E.coli</i> PBR322 KPC-2	16	≤ 0.25	0.5	1
<i>K. pneumoniae</i> (5) KPC-2	16	≤ 0.25	2	0.5
<i>K. pneumoniae</i> -ST258	128	8	32	16*
Kp animal model	32	0.5	4	0.5
Per 21	16	2	4	2

*WGS

When the antibiotic partner is cefepime (FEP) both MB_056 and MB_076 are decreasing the agar MIC's to values close to FEP and SO2030 combination.

MB076 seems to work better than MB056 for most of the strains.

Confidential

Part 4. New Directions

- MBLs
- Bisthiazolidine (BTZs), Fragment libraries (DPAs)
- New Approaches
- Back to Basics

Courage to forge new paths



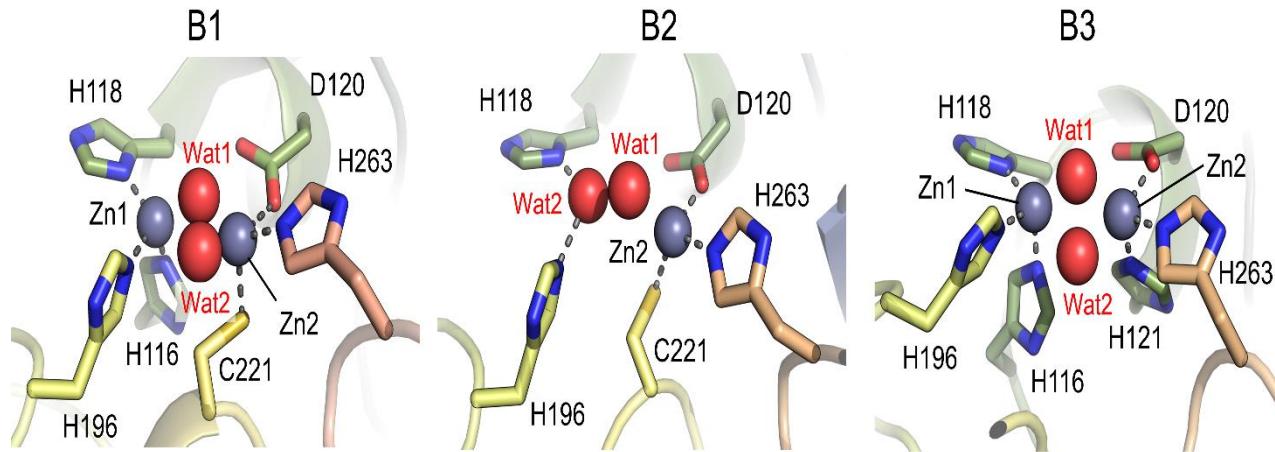
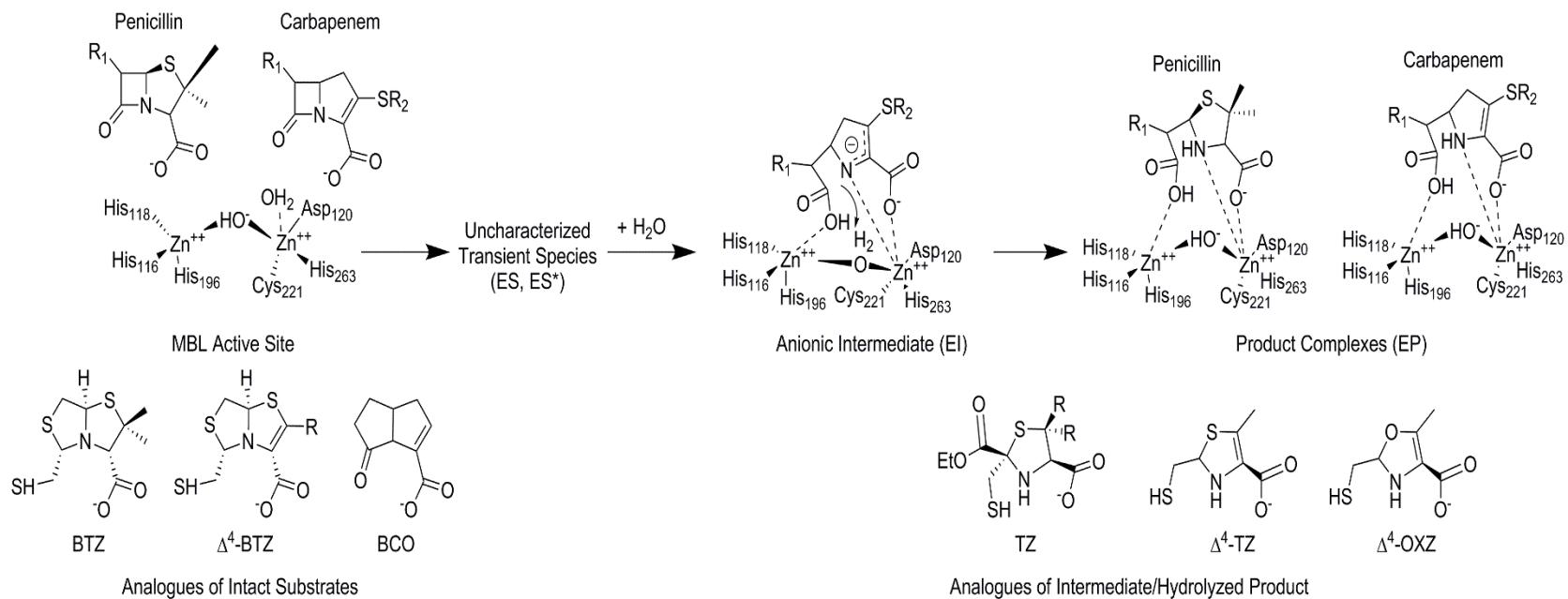
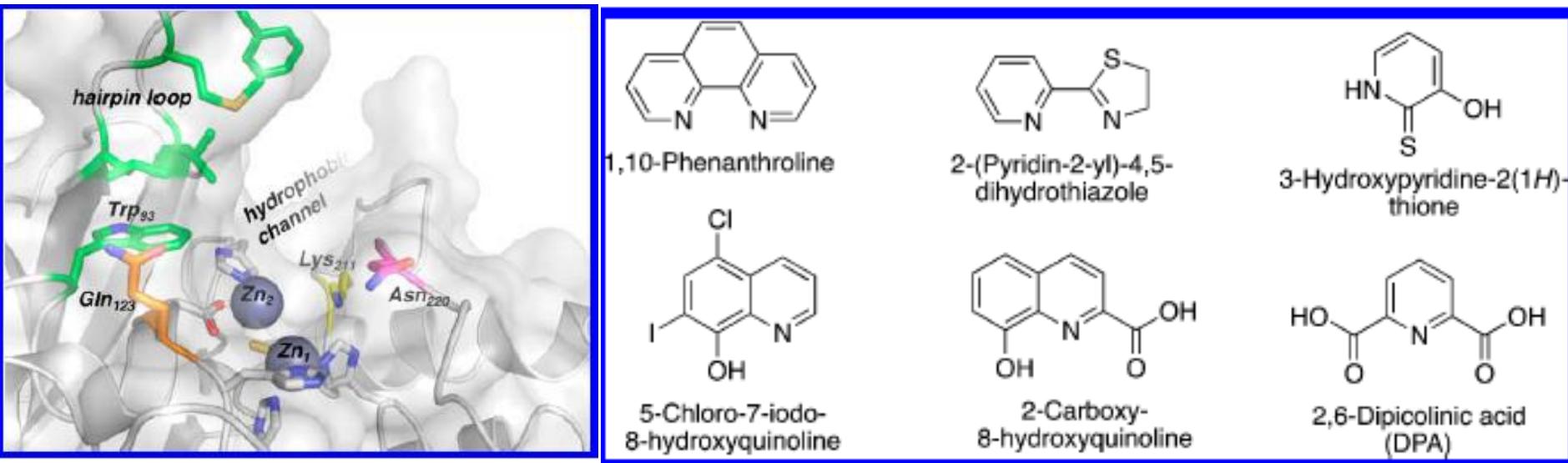


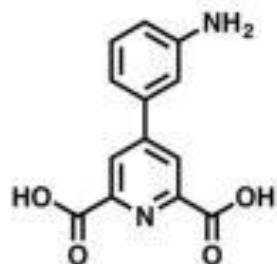
Figure 1: Active Sites of MBL Subclasses. B1 (Left); B2 (Center); B3 (Right).



Dipicolinic Acid Derivatives as Inhibitors of New Delhi Metallo- β -lactamase-1

Allie Y. Chen,[†] Pei W. Thomas,[‡] Alesha C. Stewart,[‡] Alexander Bergstrom,[§] Zishuo Cheng,[§] Callie Miller,[§] Christopher R. Bethel,^{||} Steven H. Marshall,^{||} Cy V. Credille,[†] Christopher L. Riley,[†] Richard C. Page,[§][¶] Robert A. Bonomo,^{*,||,##} Michael W. Crowder,^{*,§}[¶] David L. Tierney,^{*,§}[¶] Walter Fast,^{*,‡}[¶] and Seth M. Cohen^{*,†}[¶]





$IC_{50} = 80 \text{ nM}$

Formation of Ternary Complex
Reduced MICs Against *E. coli* and *K. pneumoniae*
Non-Cytotoxic

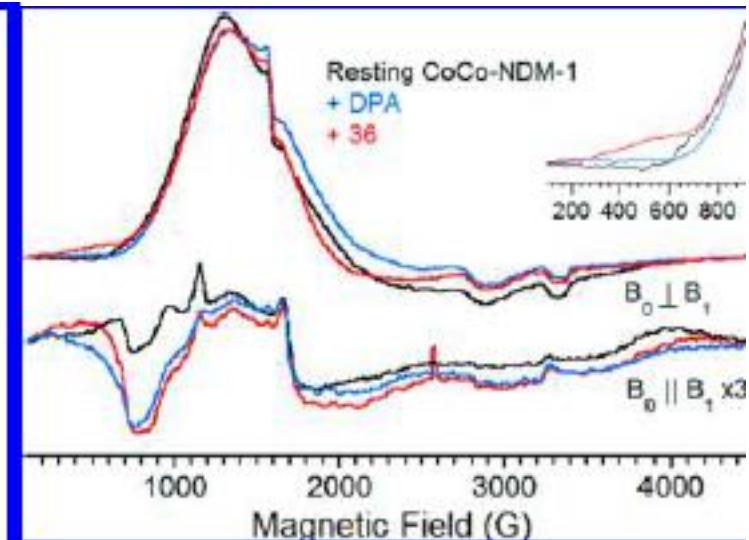
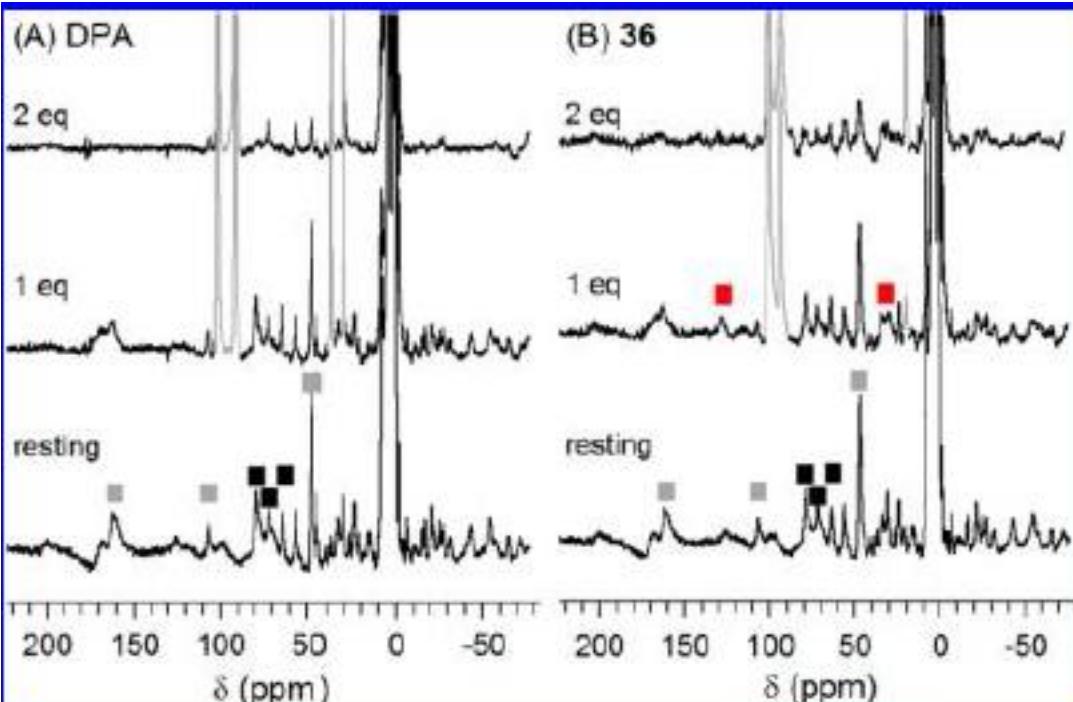
Table 4. IC_{50} Values of Selected Compounds with B1 MBLs Using Fluorocillin as a Substrate

compd	$IC_{50} (\mu\text{M})$		
	NDM-1	VIM-2	IMP-1
DPA	0.41 ± 0.02	1.66 ± 0.03	3.03 ± 0.04
36	0.080 ± 0.002	0.21 ± 0.01	0.24 ± 0.01

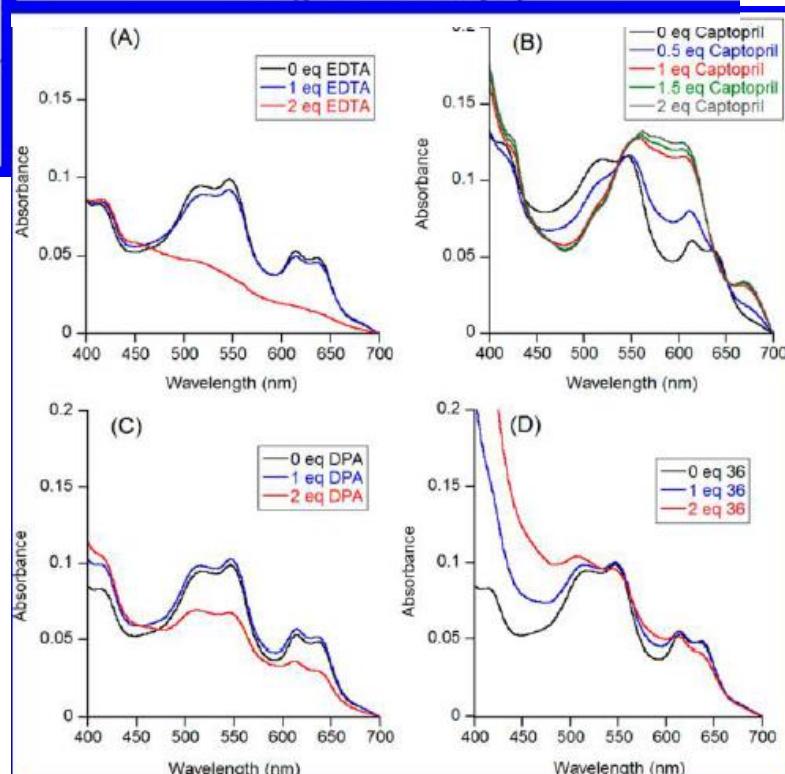
<i>E. coli</i> isolate	MIC (mg/L)	
	imipenem	imipenem + 36
Ch8.68	16	1
Ch8.69	4	0.5
Ch8.70	16	0.5
Ah8.71	16	1
Ch8.72	16	0.5
Ah8.73	16	0.5
Ah8.74	8	0.5
Ah8.75	16	0.5

<i>K. pneumoniae</i> isolate	MIC (mg/L)	
	imipenem	imipenem + 36
Pd1.48	8	0.5
Pd1.49	4	1
Pd1.50	8	0.5
Pd1.53	8	0.5
Pd1.54	8	0.5
Pd1.55	8	0.5
Cm1.62	16	1
Cm1.63	8	1

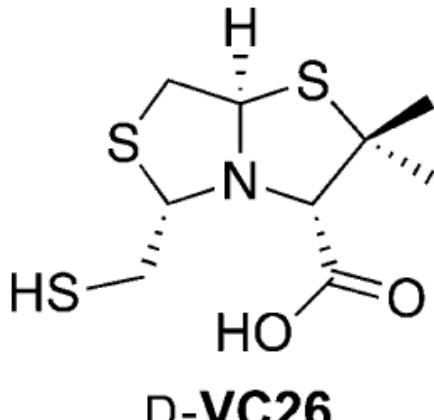
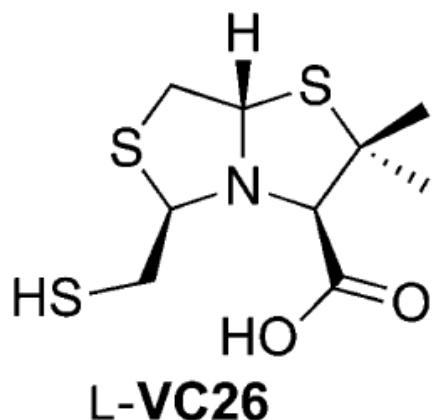
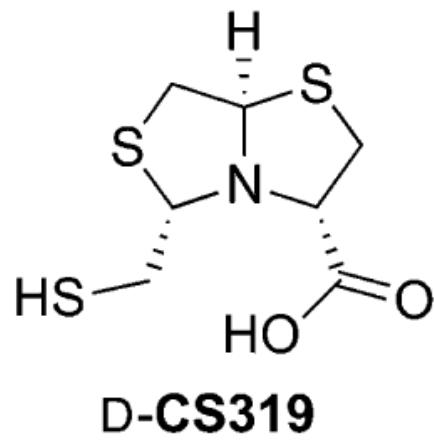
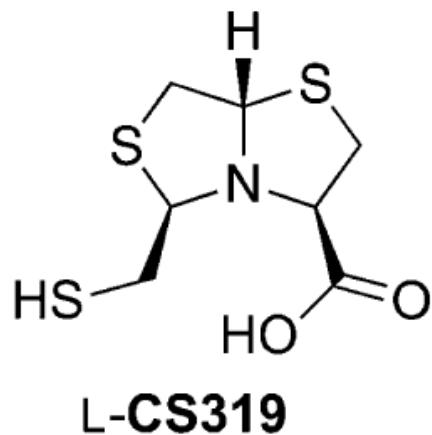
^aAll strains possess *bla*_{CTX-M-15} and *bla*_{CMY-2} except for *E. coli* Ah8.74 which possesses only *bla*_{NDM-1}.



While DPA displayed a propensity to chelate metal ions from NDM-1, 36 formed a stable NDM-1:Zn(II):inhibitor ternary complex, as demonstrated by ^1H NMR, electron paramagnetic resonance (EPR) spectroscopy, equilibrium dialysis, intrinsic tryptophan fluorescence emission, and UV-vis spectroscopy.



Bisthiazolidine (BTZs) as inhibitors



The bicyclic BTZ structure mimics the β -lactam scaffold

BTZ carboxylate is able to replicate the interactions of the equivalent group at C3/C4 of β -lactams



Catalytic mechanism for B1 MBLs

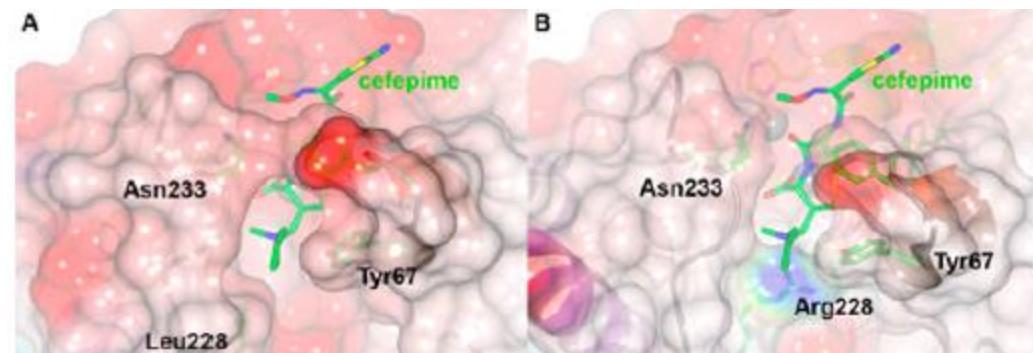
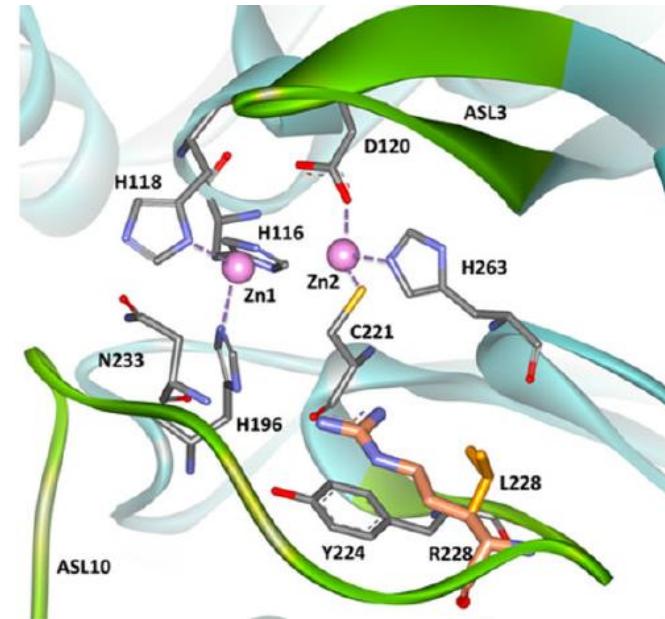
R228L MICs of cefepime from 0.5 to 4 mg/L

In some ways, this natural variant (R228L) recapitulates the clinical observation made in class A β -lactamases such as TEM or SHV, in which a single amino acid substitution results in an “extended spectrum” phenotype.

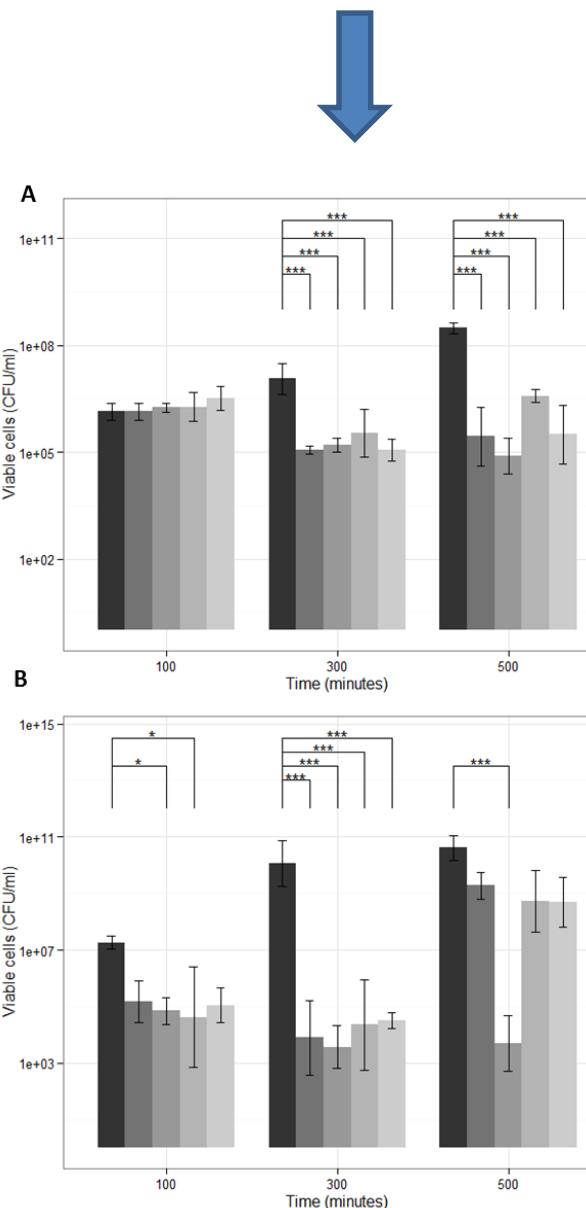


Exploring the Role of Residue 228 in Substrate and Inhibitor Recognition by VIM Metallo- β -lactamases

Maria F. Mojica^{‡,†,||}, S. Graciela Mahler[†], Christopher R. Bethel^{||}, Magdalena A. Taracila^{‡,||}, Magda Kosmopoulou^{||}, Krisztina M. Papp-Wallace^{‡,||}, Leticia I. Llarrull[#], Brigid M. Wilson^{||}, Steven H. Marshall^{||}, Christopher J. Wallace^{||}, Maria V. Villegas[†], Michael E. Harrist, Alejandro J. Vila[#], James Spencer^{||,‡}, and Robert A. Bonomo^{*†,||,§}

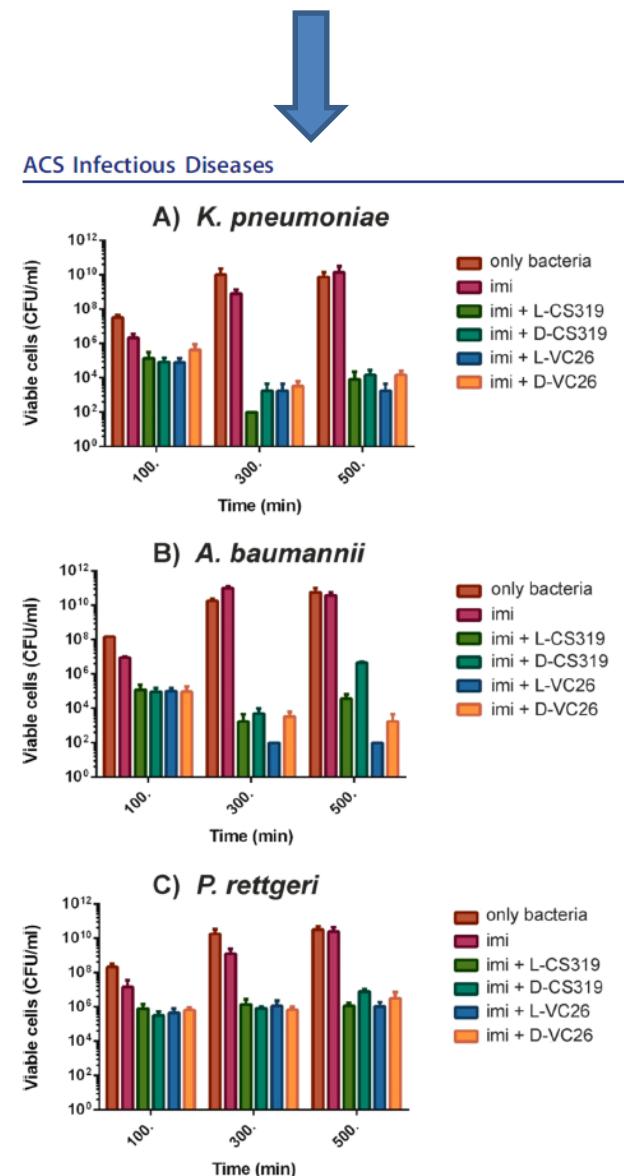


BTZs restore the *in vitro* activity of imipenem against VIM-2 producing *P. aeruginosa* (A) and VIM-24 producing *K. pneumoniae* (B).



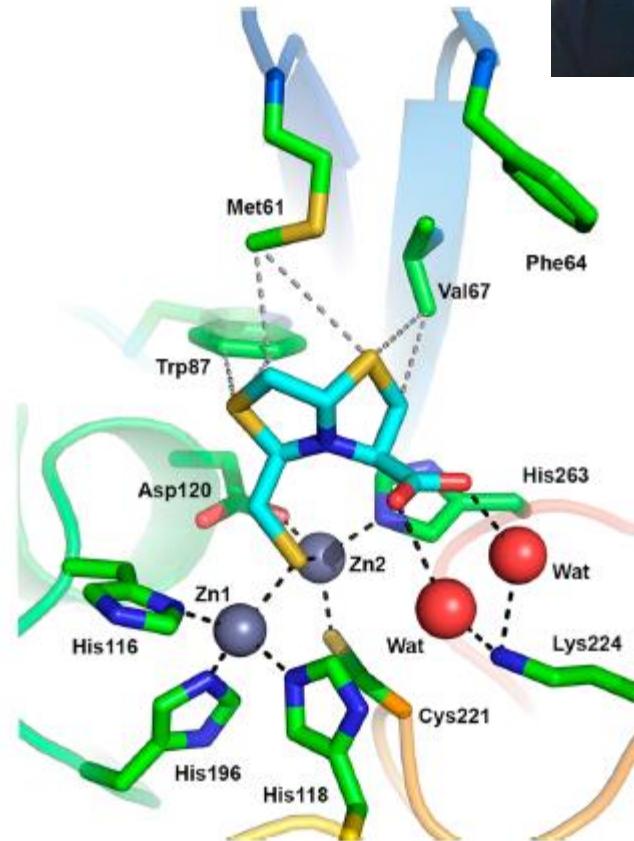
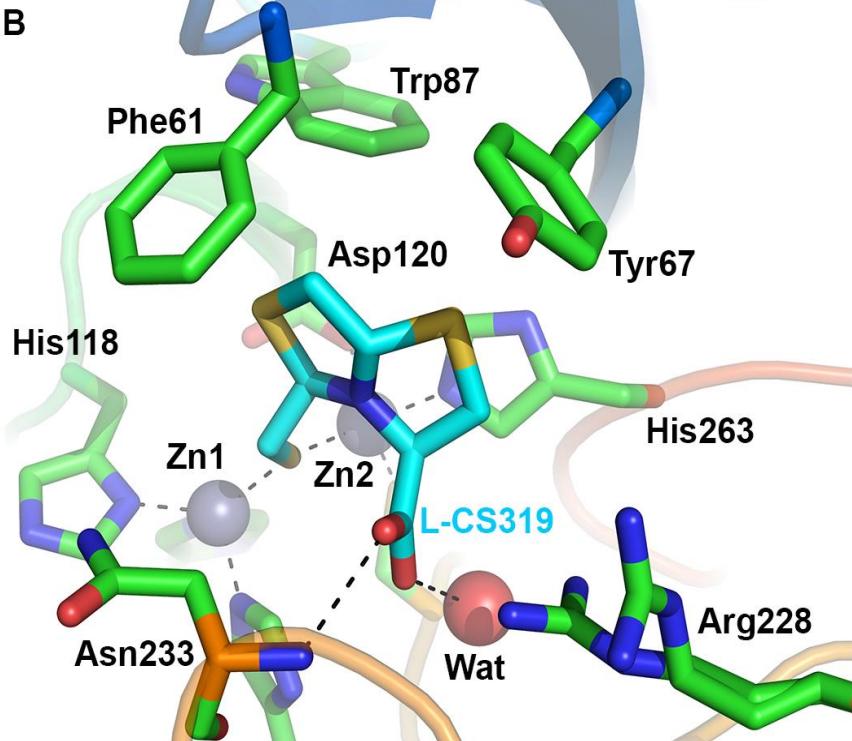
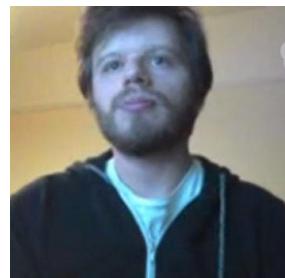
No tox against HeLa and Hek293 cells at 500 μ M

Against NDM-1-producing *K. pneumoniae* (A), *A. baumannii* (B), and *P. rettgeri* (C).



Cross-class metallo- β -lactamase inhibition by bisthiazolidines reveals multiple binding modes

Philip Hinchliffe^a, Mariano M. González^b, María F. Mojica^{c,d}, Javier M. González^e, Valerie Castillo^f, Cecilia Saiz^f, Magda Kosmopoulou^a, Catherine L. Tookey^a, Leticia I. Llarrull^b, Graciela Mahler^f, Robert A. Bonomo^{c,d,g,h,i}, Alejandro J. Vila^{b,1}, and James Spencer^{a,1}



CS319 inhibits
VIM-2 and NDM-1

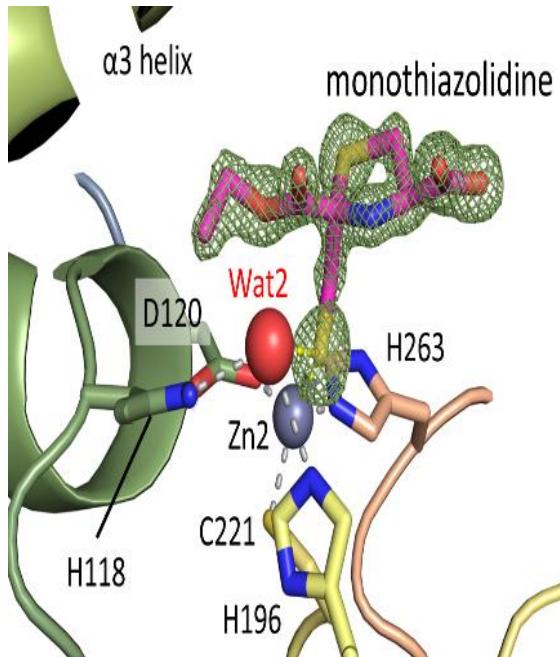


Figure 5: TZs as MBL Inhibitors. A. Structures of synthesized thiazolidines, and comparison with hydrolyzed benzylpenicillin. B. Crystal structure of Sfh-I complex with L-TZ-1 (preliminary data, J. Spencer).

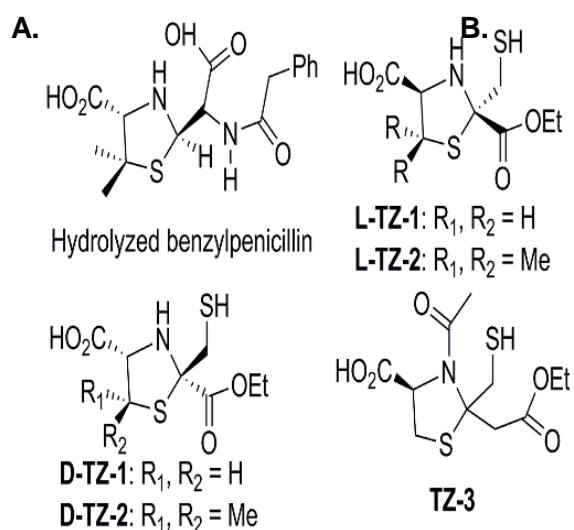
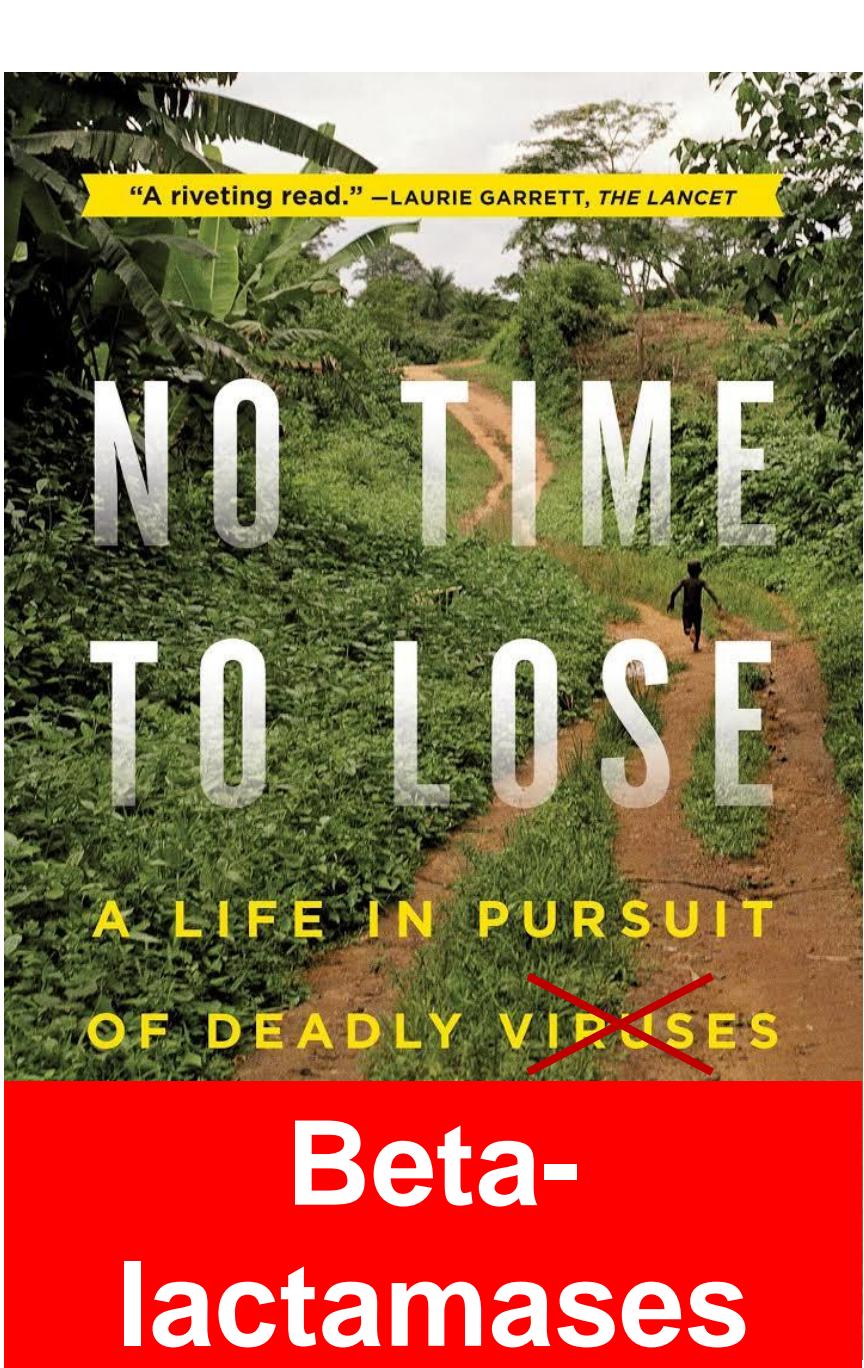


Figure 5: TZs as MBL Inhibitors. A. Structures of synthesized thiazolidines, and comparison with hydrolyzed benzylpenicillin.

Challenges ahead

- Identification of measureable biological correlates of β -lactamase inhibition in the cell are still elusive.
- New BLIs and β -lactams
- New collaborations;like getting married “right partner” is key.....“genius is knowing a good idea when you hear one”
- New ideas and new tools protein science/NMR/peptide fragments/antibody based therapies/diagnostic platforms/analytical tools/cryoEM/AFM/SEM...



"A riveting read." —LAURIE GARRETT, THE LANCET

NO TIME TO LOSE

A LIFE IN PURSUIT
OF DEADLY VIRUSES

Beta-
lactamases

**"No time to lose":
Forward looking
science for the benefit
of patients must be
based upon sound
theory and should be
our mandate – need to
marry hypothesis
testing at the bench to
the clinic**

